Table 2b : Total numbers of HGPRT-mutant cells, mutant frequency and viability of Chinese hamster V79 cells after a 5-hour exposure to RO 40-7592/001 and CM 59 MIX

Dose	Cell Viability Day 2			Day 7			HGPRT-Mutant Cells Day 7			
ug/ml	No. (a)	Mean	RV %	No. (a)	Mean	CE %	No. (b)	Mean	Sign.	MF per 10^4 cells
0.	150 176 175 187	172.0	100	185 150 205 166	176.5	88	0 0 0 0 0 0 0 0 0 1 1 2	0.3		3.8
5.	182 199 210 197	197.0	100	169 181 195 194	184.8	92	0 0 0 0 0 0 0 0 0 0 0 0	0.0		< 0.9
50.	110 118 121 129	119.5	69	148 166 189 181	171.0	86	0 0 0 0 0 0 1 1 1 2 2 3	0.8		9.7
200.	147 126 152 144	142.3	83	221 207 219 178	206.3	103	0 0 0 0 1 1 1 1 1 1 2 2	0.8		8.1
300.	162 152 149 127	147.5	86	177 194 176 167	178.5	89	0 0 0 0 0 0 0 0 0 0 0 2	0.2		1.9
400.	33 33 39 35	35.0	20	101 101 106 85	98.3	49	0 0 0 0 0 0 0 0 0 0 0 1	0.1		1.7
Reference Substance : 2AAF										
0.	150 176 175 187	172.0	100	185 150 205 166	176.5	88	0 0 0 0 0 0 0 0 0 1 1 2	0.3		3.8
60.	107 105 104 79	98.8	57	131 140 141 140	138.0	69	5 6 5 8 8 9 9 9 9 10 12 15	8.6		124.4

Experiment Number : 22 M 90/2

a: 200 cells were plated per dish
b: 10^5 cells were plated per dish

* for P =< 0.05 ** for P =< 0.01

Trend: (+) increasing / (-) decreasing

C.5.e. Unscheduled DNA Synthesis with Tolcapone in Primary Cultures of Rat Hepatocytes

Research Report #: B-154,906
Sponsor Volume: 51:91

Summary:

The effect of tolcapone on unscheduled DNA synthesis was determined in primary cultures of rat hepatocytes. Only a narrow concentration of range 1-5 µg/ml was tested, since concentrations higher than 5 µg/ml were toxic. In one of three trials, concentrations of 1 and 4 µg/ml significantly increased nuclear grain counts, but a concentration-effect relationship was not evident; this is considered a spurious finding. The positive control 2-acetylaminofluorene produced the expected result.

Methods:

Drug Concentrations: Batch G PUL 557 089 in DMSO

Test Chemical	Substance Concentration µg/ml		
	23M90/0	/1	/2
Solvent control: DMSO	0.0	0.0	0.0
Ro 40-7592/001	1.0	1.0	0.5
Ro 40-7592/001	5.0	5.0[1]	1.0
Ro 40-7592/001	10.0[2]	10.0[2]	2.0
Ro 40-7592/001	25.0[2]	25.0[2]	3.0
Ro 40-7592/001	50.0[2]	50.0[2]	4.0[1]
Ro 40-7592/001	75.0[2]	75.0[2]	5.0[1]
Ro 40-7592/001	100.0[2]	100.0[2]	
Reference substance: 2AAF	0.1	0.1	0.1[1]

[1] Cell morphology of many cells had changed at this dose level
[2] No viable cells available

Experimental Procedure:

Rat hepatocytes were isolated by *in situ* liver perfusion of adult male Fu-albino rats on the day of experiment. Cells were seeded into plates containing a plastic coverslip for cell attachment at a density 1.5 x 10^5 cells/plate. Four coverslips per dose were used to measure [^{3}H]-thymidine incorporation, and one coverslip/dose was used to assess cell viability trypan blue exclusion. Additional coversliups were used to determine cell morphology and viability after exposure to test compounds.

The UDS assay was initiated within 5 hrs of cell seeding, by replacing the medium with medium containing [^{3}H]-thymidine and the test compound. Exposures were for 18 hrs, and followed by washing,. fixing, and rinsing. [^{3}H]-Thymidine incorporation was determined autoradiographically by counting the number of silver grains in nuclei of non-replicating cells. Usually 100 cells that were not in S phase per dose (ca. 25/coverslip) were counted. Cytotoxicity was assessed by counting non-nuclear silver grains, and determination of net nuclear grains.

Statistics

Nuclear grain counts, cytoplasmic grain counts, and net nuclear grain counts were evaluated by one-way ANOVA with a post-hoc Fisher's least significant difference test ($p<0.05$).

Results:

Significant increases in nuclear grain counts in primary rat hepatocytes were observed only in the third experimental trial at concentrations of 1 and 4 µg/ml (Table 1c). In two previous experiments with concentrations of 1 and 5 µg/ml, no increase in nuclear grain counts were observed (Tables 1a and 1b).

Table 1a : Unscheduled DNA Synthesis (UDS) assay with freshly isolated rat hepatocytes after 18-hours exposure to Ro 40-7592/001 (Experiment 23M90/0)

Test Chemical	Dose µg/ml	No.Cell Analysed	Grain Counts / Cell			
			Nuclear > 5 (%)	Nuclear Mean ± SD	Cytoplasmic Mean ± SD	Net Nuclear Mean ± SD
Negative control: DMSO	0.0	100	0	1.4 ± 1.2	1.9 ± 1.3	-0.5 ± 1.5
Ro 40-7592/001	1.0	100	1	1.3 ± 1.2	1.8 ± 1.1	-0.5 ± 1.1
Ro 40-7592/001	5.0	50	0	1.1 ± 1.0	1.4 ± 0.9	-0.4 ± 1.1
Reference substance: 2AAF	0.1	94	67	8.1 ± 4.9	2.1 ± 1.3	6.0 ± 4.4

Statistical significance: * for $p \leq 0.05$, ** for $p \leq 0.01$

Table 1b : Unscheduled DNA Synthesis (UDS) assay with freshly isolated rat hepatocytes after 18-hours exposure to Ro 40-7592/001 (Experiment 23M90/1)

Test Chemical	Dose µg/ml	No.Cell Analysed	Grain Counts / Cell			
			Nuclear > 5 (%)	Nuclear Mean ± SD	Cytoplasmic Mean ± SD	Net Nuclear Mean ± SD
Negative control: DMSO	0.0	75	0	1.0 ± 1.0	1.2 ± 0.8	-0.2 ± 1.1
Ro 40-7592/001	1.0	90	2	1.5 ± 1.4	1.8 ± 1.2	-0.3 ± 1.2
Ro 40-7592/001	5.0	100	1	0.7 ± 1.1	0.9 ± 0.9	-0.1 ± 0.9
Reference substance: 2AAF	0.1	80	63	7.5 ± 4.4	2.9 ± 1.9	4.6 ± 4.0

Statistical significance: * for $p \leq 0.05$, ** for $p \leq 0.01$

Table 1c : Unscheduled DNA Synthesis (UDS) assay with freshly isolated rat hepatocytes after 18-hours exposure to Ro 40-7592/001 (Experiment 23M90/2)

Test Chemical	Dose µg/ml	No.Cell Analysed	Grain Counts / Cell			
			Nuclear > 5 (%)	Nuclear Mean ± SD	Cytoplasmic Mean ± SD	Net Nuclear Mean ± SD
Negative control: DMSO	0.0	102	0	0.3 ± 0.6	0.5 ± 0.5	-0.1 ± 0.5
Ro 40-7592/001	0.5	101	0	0.6 ± 0.9	0.9 ± 0.7	-0.3 ± 0.9
Ro 40-7592/001	1.0	104	0	0.8* ± 0.9	0.8 ± 0.9	-0.1 ± 0.9
Ro 40-7592/001	2.0	100	0	0.4 ± 0.7	0.4 ± 0.5	0.01 ± 0.7
Ro 40-7592/001	3.0	101	0	0.5 ± 0.8	0.6 ± 0.7	-0.1 ± 0.8
Ro 40-7592/001	4.0	100	0	0.9* ± 1.0	0.9 ± 0.6	0.02 ± 0.9
Ro 40-7592/001	5.0	101	0	0.5 ± 0.6	0.7 ± 0.6	-0.2 ± 0.7
Reference substance : 2AAF	0.1	101	30	5.0 ± 2.8	2.0 ± 1.2	3.0 ± 2.3

Statistical significance: * for $p \leq 0.05$, ** for $p < 0.01$

Cell viability at concentrations up to 5 µg/ml was 74-90%. The next higher test concentration of 10 µg/ml was toxic and resulted in complete loss of viability (Tables 2a-c).

Table 2a : Viability of rat hepatocytes after 18 hours exposure to Ro 40-7592/001 (Exp.23M90/0)

Test Chemical	Dose µg/ml	Cell Viability[1] %	Relative Viability %
Negative control	0.0	97	100
Ro 40-7592	1.0	97	100
Ro 40-7592	5.0	90	93
Ro 40-7592	10.0	No viable cells available	
Ro 40-7592	25.0	No viable cells available	
Ro 40-7592	50.0	No viable cells available	
Ro 40-7592	75.0	No viable cells available	
Ro 40-7592	100.0	No viable cells available	
Reference: 2AAF	0.1	89	92

[1] As measured by the method of in situ dye exclusion

Table 2b : Viability of rat hepatocytes after 18 hours exposure to Ro 40-7592/001 (Exp.23M90/1)

Test Chemical	Dose µg/ml	Cell Viability[1] %	Relative Viability %
Negative control	0.0	91	100
Ro 40-7592	1.0	83	91
Ro 40-7592	5.0	77[2]	85
Ro 40-7592	10.0	No viable cells available	
Ro 40-7592	25.0	No viable cells available	
Ro 40-7592	50.0	No viable cells available	
Ro 40-7592	75.0	No viable cells available	
Ro 40-7592	100.0	No viable cells available	
Reference: 2AAF	0.1	87	96

[1] As measured by the method of in situ dye exclusion
[2] Morphology of many cells had changed at this dose level

Table 2c : Viability of rat hepatocytes after 18 hours exposure to Ro 40-7592/001 (Exp.23M90/2)

Test Chemical	Dose µg/ml	Cell Viability[1] %	Relative Viability %
Negative control	0.0	85	100
Ro 40-7592	0.5	84	99
Ro 40-7592	1.0	75	88
Ro 40-7592	2.0	83	98
Ro 40-7592	3.0	84	99
Ro 40-7592	4.0	83[2]	98
Ro 40-7592	5.0	74[2]	87
Reference: 2AAF	0.1	85[2]	100

[1] As measured by the method of in situ dye exclusion
[2] Cell morphology had changed slightly at this dose level

C.5.f. Chromosome Analysis in Human Lymphocytes Exposed to Tolcapone *In Vitro*

Research Report #: B-154,840

Sponsor Volume: 51

Summary:

Cultures of human lymphocytes were exposed to tolcapone in the presence and absence of S9 metabolizing fractions. Chromosomal damage was not detected under any test condition.

This assay did not meet the OECD guidelines as only two analyzable concentrations were used.

Methods:

Drug Concentrations and Exposures:	Batch G PUL 493 089	
Without S9 activation:	100 - 400 µg/ml;	3 hr exposure
	5 - 40 µg/ml;	24 hr exposure
	5 - 30 µg/ml;	46 hr exposure
With S9 activation:	50 - 400 µg/ml;	3 hr exposure
Positive Controls/vehicles:		
Without S9 activation:	Bleomycin (5 - 50 µg/ml) in water	
With S9 activation:	Cyclophosphamide (20 - 30 µg/ml) in water	

Test System:

The following is the reviewer's interpretation of a rather confusing description of the experimental methods. It was not clear if or when PHA was added to "long-term" treatment cultures without metbaolic activation. The use of the terms "incubation" in the text and "recovery" in the tables, and the length of these incubations or recoveries were also difficult to decipher. Thus, it is possible that some discrepancies may exist between the description below and the actusl methods.

Microcultures of whole human blood were prepared and stimulate to divide by addition of phytohemagglutinin. After a 24 hr incubation, cells were harvested and resuspended in medium containing different dilutions of tolcapone or the control compounds (in triplicate). For the short-term exposure experiments, the cells were incubated with tolcapone (or controls) with or without S9 fraction for 3 hrs, followed by a change of medium containing bromodeoxyuridine and PHA. Incubations were continued for 21 or 43 hr (24 or 46 hr recovery). For the long-term drug exposures, which were not carried out with S9 activation, the bromodeoxyuridine and PHA (see above) was added together with the test substance. Incubations were continued for 24 or 46 hr.

At 3 hr before harvest, colchicine was added to the cultures to arrest cells in metaphase. After harvest, the cells were fixed in methanol/acetic acid, suspended in fixative, and mounted on slides. Four slides per culture were prepared without BrdU and 2 slides per culture were prepared with BrdU. On the following day, cells without BrdU were incubated with 1N HCl, and cells with BrdU were incubated with bis-benzimide. Slides were then stained with Giemsa, and analyzed for metaphases resulting from the second mitosis after treatment.

The test concentration range was established by determining the mitotic index in cultures (percent of cells in mitosis).

Metabolizing System:

S9 fraction prepared from Arochlor-induced rats (500 mg/kg).

Statistics: Fisher's Exact test ($p<0.05$; two-tailed test)

Results:

Short-term (3 hr) incubations of cultured human lymphocytes with 400 µg/ml tolcapone in the absence (Table 1) or presence of S9 (Table 2) resulted in cytotoxicity. The two lower concentrations (100, 200 µg/ml) had no effect on mitotic index or chromosomal abberations.

12. Summary tables

Table 1: Rate of chromosome damage, index of cells with structural aberrations (S-cells), with unspecific chromosome changes (U-cells) and of cells in mitosis (M-I) in cultured human lymphocytes treated for 3 h without metabolic activation (recovery 24 h).

Test Substance	Dose µg/ml	Analysed Cells	M-I %	S-Cells N	S-Cells %	CHRI	EX	ACE	R_C	DIC	ATYP	P	U-Cells N	U-Cells %	GAPS
018 M 90/1															
Negative control	0	100	4.6	0	0.00								2	2.00	0.020
Solvent control	0	100	3.5	1	1.00			0.010					0	0.00	0.010
Concurrent negativ controls	0	200	4.1	1	0.50			0.005					2	1.00	0.015
Ro 40-7592/001	100	200	4.6	1	0.50					0.005			6	3.00	0.030
"	200	200	8.4	1	0.50			0.005					3	1.50	0.020
"	400	0													
Positive control:															
Bleomycin	50	100	2.9	29	29.00**	0.150	0.010	0.130		0.110	0.030		10	10.00**	0.340

* Significant at the 5 % level
** Significant at the 1 % level

Table 2: Rate of chromosome damage, index of cells with structural aberrations (S-cells), with unspecific chromosome changes (U-cells) and of cells in mitosis (M-I) in cultured human lymphocytes treated for 3 h with metabolic activation (recovery 24 h).

Test Substance	Dose µg/ml	Analysed Cells	M-I %	S-Cells N	S-Cells %	CHRI	EX	ACE	R_g	DEC	MTTP	P	U-Cells N	U-Cells %	GAPS
018 M 90/1															
Negative control	0	100	4.6	0	0.00								2	2.00	0.020
Solvent Control	0	100	3.5	1	1.00			0.010					0	0.00	0.010
S-9 control	0	100	5.4	2	2.00		0.010	0.010					1	1.00	0.010
Concurrent negative controls	0	300	4.5	3	1.00		0.003	0.007					3	1.00	0.013
Ro 40-7392/001	100	200	5.2	3	1.50	0.005		0.005		0.005	0.005		1	0.50	0.005
"	200	200	3.5	1	0.50			0.005					3	1.50	0.015
"	400	0	0.0												
Positive control:															
Cyclophosphamid	20	100	4.2	11	11.00**	0.020		0.100		0.010			1	1.00	0.030
	30	100	0.5	15	16.00**	0.020	0.010	0.110			0.030		10	10.00**	0.120

* Significant at the 5 % level
** Significant at the 1 % level

Longer term incubations (24 or 46 hrs) with tolcapone in the absence of S9 caused a marked cytoxicity at concentrations of 50 and 30 µg/ml (Tables 3 and 4). The two lower concentrations had no effect on mitotic index or chromosomal abberations.

Table 3: Rate of chromosome damage, index of cells with structural aberrations (S-cells), with unspecific chromosome changes (U-cells) and of cells in mitosis (M-I) in cultured human lymphocytes treated for 24 h without metabolic activation.

Test Substance	Dose µg/ml	Analysed Cells	M-I %	S-Cells N	S-Cells %	CHRI	EX	ACE	R_g	DEC	ATTP	P MA	U-Cells N	U-Cells %	GAPS
018 M 90/4															
Negative control	0	200	9.7	1	0.50			0.005					2	1.00	0.010
Solvent control	0	200	8.6	1	0.50						0.005		3	1.50	0.015
Concurrent negativ controls	0	400	9.2	2	0.50			0.003			0.003		5	1.25	0.013
Ro 40-7592/001	10	200	6.4	1	0.50			0.005					1	0.50	0.005
"	25	200	4.9	3	1.50		0.005	0.010					5	2.50	0.025
"	50	200	1.4	1	0.50					0.005			6	3.00	0.030
Positive control:															
Bleomycin	5	100	5.4	27	27.00**	0.200	0.020	0.190		0.030	0.010		13	13.00**	0.290

* Significant at the 5 % level
** Significant at the 1 % level

Table 4: Rate of chromosome damage, index of cells with structural aberrations (S-cells), with unspecific chromosome changes (U-cells) and of cells in mitosis (M-I) in cultured human lymphocytes treated for 46 h without metabolic activation.

Test Substance	Dose µg/ml	Analysed Cells	M-I %	S-Cells N	S-Cells %	CHRI	EX	ACE	R_g	DEC	ATTP	P MA	U-Cells N	U-Cells %	GAPS
018 M 90/2															
Negative control	0	100	8.5	1	1.00	0.010							1	1.00	0.010
Solvent control	0	100	5.4	2	2.00			0.020					6	6.00	0.060
Concurrent negativ controls	0	200	7.0	3	1.50	0.005		0.010					7	3.50	0.035
Ro 40-7592/001	5	200	4.9	3	1.50	0.005		0.005		0.005	0.005		3	1.50	0.015
"	10	200	5.2	1	0.50			0.005					2	1.00	0.010
"	30	134	0.5	6	4.41	0.015	0.007	0.015			0.007		4	2.94	0.044
Positive control:															
Bleomycin	5	100	2.9	42	42.00**	0.220	0.030	0.050	0.010	0.060		4 11	19	19.00**	0.810

* Significant at the 5 % level
** Significant at the 1 % level

To test for possible longer term effects of metabolic activation, cultures were exposed to S9 for 3 hr then allowed to recover for 46 or 24 hr. In these experiments, cytotoxicity or chromosomal abberations were not evident at concentrations up to 300 or 400 µg/ml (Tables 5 and 6).

Table 5: Rate of chromosome damage, index of cells with structural aberrations (S-cells), with unspecific chromosome changes (U-cells) and of cells in mitosis (M-I) in cultured human lymphocytes treated for 3 h with metabolic activation (recovery 46 h).

Test Substance	Dose µg/ml	Analysed Cells	M-I %	S-Cells N	S-Cells %	CHRI	EX	ACE	R_c	DEC	ATTP	P	U-Cells N	U-Cells %	GAPS
010 M 90/2															
Negative control	0	100	8.5	1	1.00	0.010							1	1.00	0.010
Solvent Control	0	100	8.4	2	2.00			0.020					6	6.00	0.060
S-9 control	0	100	9.1	2	2.00			0.020					4	4.00	0.040
Concurrent negative controls	0	300	7.7	5	1.67	0.003		0.013					11	3.67	0.037
Ro 40-7592/001	50	200	7.4	4	2.00			0.020					3	1.50	0.020
"	150	200	10.1	4	2.00	0.005		0.025					2	1.00	0.010
"	300	200	8.3	4	2.00			0.020					6	3.00	0.030
Positive control:															
Cyclophosphamid	20	100	6.5	6	6.00*	0.010		0.040			0.010		2	2.00	0.020
"	30	100	5.6	9	9.00**			0.100					7	7.00	0.100

Table 6 : Rate of chromosome damage, index of cells with structural aberrations (S-cells), with unspecific chromosome changes (U-cells) and of cells in mitosis (M-I) in cultured human lymphocytes treated for 3 h with metabolic activation (recovery 24 h).

Test Substance	Dose µg/ml	Analysed Cells	M-I %	S-Cells N	S-Cells %	CHRI	EX	ACE	R_c	DEC	ATTP	P NA	U-Cells N	U-Cells %	GAPS
010 M 90/4															
Negative control	0	200	9.7	1	0.50			0.005					2	1.00	0.010
Solvent control	0	200	8.6	1	0.50						0.005		3	1.50	0.015
Concurrent negativ controls	0	400	9.2	2	0.50			0.003			0.003		5	1.25	0.013
Ro 40-7592/001	300	200	9.9	4	2.00	0.010		0.010		0.005			3	1.50	0.015
"	400	200	12.1	3	1.50	0.005		0.010		0.005			5	2.50	0.025
Positive control:															
Cyclophosphamid	20	100	2.5	69	69.00**	0.540	0.380	0.510	0.010		0.010	0 13	15	15.00**	0.660

* Significant at the 5 % level
** Significant at the 1 % level

All control samples generally produced the expected results. In the experiment with 3 hr metabolic activation and a 46 hr recovery period, cyclophosphamide did not produce as dramatic an increase in number of S-phase cells as was noted with positive controls in other experiments.

It should be noted that in most of these experiments only two concentrations could be considered analyzable because of the sharp decrease in mitotic index between the intermediate and high test concentrations.

C.5.g. Mutagenicity of Tolcapone in Combination with Sinemet in the ML/TK Assay

Research Report #: B-163,208
Sponsor Volume: 51

Summary:

Mutagenic properties of tolcapone in combination with Sinemet were evaluated in the mouse lymphoma/thymidine kinase gene mutation assay. Positive effects, particularly elevations in the freqency of small colony formation were identified, and attributed to tolcapone.

In the absence of S9, a small but statistically significant increase (1.6X) in **total** colony formation at a combined drug concentration of 200 µg/ml was reported by the sponsor (Table 4.c.). A more marked, dose-related increase (3.2X at 200 µg/ml) in **small** colony formation was not commented upon by the sponsor. When the compounds were tested separately, no statistically significant effects on **total** colony formation were detected by the sponsor. However, the concentration of 100 µg/ml tolcapone alone caused an approximate 2-fold increase in the appearance of **small** colonies, which also was not commented on by the sponsor. These data suggest that tolcapone is weakly mutagenic in the absence of S9 activation in the ML/TK assay. The mutagenic effects of tolcapone are primarily manifested as a selective increase the formation of **small** colonies.

In the presence of S9, clear, reproducible dose-related increases in mutant frequencies resulted from treatment of the cultures with the combination of tolcapone and Sinemet. In the main experiment (Table 4.d.), the combined drug concentration of 250 µg/ml caused a 2.2-fold increase in **total** colony formation. Again more substantial elevations in the appearance of **small** colonies was noted (4-5X). Combined drug concentrations as low as 90 µg/ml produced a 3-3.6 fold increase in **small** colony formation when relative cell viability was 70-74%. Analysis of the drugs individually suggested that the mutagenicity was due to tolcapone. The source of S9 fraction did not affect the mutagenic properties of tolcapone in this assay, as relatively similar results were obtained with S9 fractions from naive rats, or rats induced with phenobarbital/β-naphthaflavone or Arochlor.

The sponsor concludes that the toxicological relevance of the mutagenicity findings are doubtful since the increase in mutant frequency occurred only at toxic concentrations and the effect was marginal. The bases for this conclusion are unclear since the sponsor did not clearly define the level of cell viability that is considered toxic in their laboratory, or the elevations of mutant frequency that are considered toxicologically relevant. Moreover, the sponsor's analyses appear to consider only **total** colony formation; tolcapone appeared to have more significant effects (both biologically and statistically) on **small** colony formation (NOTE: statistically significant effects are identified only in the text and are not marked as such in the tables). With these considerations in mind, the aforementioned 3-3.6 fold increase in **small** colony formation at a combined drug concentration of 90 µg/ml when cell viability was 70-74% contradicts the sponsor's contention that increases in mutant frequency only occur at toxic concentrations. In addition, the effect was clearly dose-related in this experiment (4-5X increases in **small** colony formation at 250 µg/ml), and the relative cell survival rates in these experiments were higher than those considered cytotoxic by established guidelines (OECD, 10%). Thus, the sponsor's contention that the findings are "equivocal" and their "toxicological relevance... is doubtful" is disputable. In the opinion of the reviewer, the data suggest that **tolcapone is weakly mutagenic in the absence of metabolic activation**, and **mutagenic in the presence metabolic activation** in the ML/TK assay.

Methods:

Drug Concentrations:

Tolcapone (Lot 40802440) was applied combination with Sinemet (4:1 ratio of L-DOPA and carbidopa) in a 1:1 ratio to achieve the final concentrations as follows:

a. without metabolic activation

Protocol No.: 090M95/3, 090M95/5 and 090M95/7

Substance	Substance Concentration [µg/ml]		
	Range finder 090M95/3	Experiment 1 090M95/5	Experiment 2 090M95/7
Negative control: RPMI-5	0	0	0
Negative control: DMSO		0	0
Tolcapone/Sinemet	5	6.25	40
Tolcapone/Sinemet	10	12.5	40
Tolcapone/Sinemet	50	25	70
Tolcapone/Sinemet	100	50	70
Tolcapone/Sinemet	150	100	120
Tolcapone/Sinemet	300	150	120
Tolcapone/Sinemet			200
Tolcapone/Sinemet			200
Sinemet		25	100
Sinemet		75	100
Tolcapone			100
Tolcapone			100
Reference substance: NOO	—	0.1	0.1

b. with metabolic activation

Protocol No.: 090M95/2, 090M95/4 and 090M95/6

Substance	Substance Concentration [µg/ml]		
	Range finder 090M95/2	Experiment 1 090M95/4	Experiment 2 090M95/6
Negative control: RPMI-5	0	0	0
Negative control: DMSO		0	0
Tolcapone/Sinemet	5	12.5	50
Tolcapone/Sinemet	10	25	50
Tolcapone/Sinemet	50	50	90
Tolcapone/Sinemet	100	100	90
Tolcapone/Sinemet	200	200	150
Tolcapone/Sinemet	300	300	150
Tolcapone/Sinemet	500		250
Tolcapone/Sinemet	1000		250
Sinemet		50	125
Sinemet		150	125
Tolcapone			125
Tolcapone			125
Reference substance: BP	—	2	2

In a follow-up study assessing the mutagenic effects of tolcapone alone with metabolic activation, the following concentrations were applied:

c. Tolcapone with metabolic activation (phenobarbital/β-naphthoflavone induced, aroclor induced, uninduced)
Protocol No.: 090M95/9, 090M95/10, 090M95/11 and 090M95/12

Substance	Substance Concentration [µg/ml]			
	Experiment 3 090M95/9	Range finder 090M95/10	Experiment 4 090M95/11	Experiment 5 090M95/12
Negative control: DMSO	0	0	0	0
Negative control: DMSO				
Tolcapone	50	12.5	15	15
Tolcapone	75	25	20	20
Tolcapone	100	50	30	30
Tolcapone	125	100	50	50
Tolcapone			75	75
Reference substance: BP	2	—	2	2

Positive Controls:

without activation	-	4-nitroquinoline-1-oxide
with activation	-	benzo(a)pyrene

Metabolic Activation:

Initial experiments on the drug combination used microsomes from phenobarbital/β-naphthaflavone-induced rats. In follow-up studies of tolcapone alone, microsomes from Arochlor-induced and uninduced rats were also used.

Experimental Procedure:

Cells (10^7) were seeded in culture flasks and incubated for 3 days prior to the experiment. In the main experiments, cultures were run in duplicate. Single cultures were used in the preliminary experiments and tests of different S9 fractions. Cultures were incubated with test compounds in the presence or absence of S9 activation for 3 hrs, washed and resuspended, and transferred to new flasks for a two-day expression period. Cells were then transferred to 96-well titer plates and exposed to 5-trifluorothymidine. The incubation period was approximately 10 days until scorable for large and small colonies.

Cytotoxicity was determined by visualization of colony-forming units at day 2 (end of expression period) on microtiter plates prepared from cultures treated with drugs on day 0.

Statistics

Mutant frequencies of test and control groups were evaluated by Dunnett's test (assuming one-way ANOVA) after logarithmic transformation. Linear trends were evaluated by Chi-Square analysis.

Results

Cytotoxicity Testing:

The combination of tolcapone/Sinemet was cytotoxic (ca. 80% decrease in cell viability) at concentrationsof 150 µg/ml in the absence of S9, and 200 µg/ml in the presence of S9.

Table 3.a : Experiment 090M95/3

Cytotoxicity in the range-finder experiment : cell counts and viability directly after a 3 h treatment with Tolcapone/Sinemet without metabolic activation.

Test article	Conc. (µg/ml)	CC[1]	RCC (%)	EW	PE (%)	RS (%)
RPMI	0	5.5×10^6	100	21	95	100
Tolcapone/Sinemet	5	5.5×10^6	100	25	84	88
Tolcapone/Sinemet	10	5.5×10^6	100	22	92	97
Tolcapone/Sinemet	50	4.6×10^6	84	25	84	88
Tolcapone/Sinemet	100	4.5×10^6	82	63	26	27
Tolcapone/Sinemet	150	4.5×10^6	82	69	21	22
Tolcapone/Sinemet	300	2.3×10^6	42	96	0	0

[1] 5×10^6 cells seeded per flask

Table 3.b : Experiment 090M95/2

Cytotoxicity in the range-finder experiment : cell counts and viability directly after a 3 h treatment with Tolcapone/Sinemet with metabolic activation.

Test article	Conc. (µg/ml)	CC[1]	RCC (%)	EW	PE (%)	RS (%)
RPMI	0	4.4×10^6	100	23	89	100
Tolcapone/Sinemet	5	5.5×10^6	125	27	79	89
Tolcapone/Sinemet	10	5.4×10^6	123	25	84	94
Tolcapone/Sinemet	50	5.1×10^6	116	25	84	94
Tolcapone/Sinemet	100	5.1×10^6	116	44	49	55
Tolcapone/Sinemet	200	3.8×10^6	86	71	19	21
Tolcapone/Sinemet	300	2.1×10^6	48	70	20	22
Tolcapone/Sinemet	500	0.5×10^6	11	96	0	0
Tolcapone/Sinemet	1000	0.5×10^6	11	96	0	0

[1] 5×10^6 cells seeded per flask

The range-finder experiments 090M95/1 and 090M95/0 were not included in the report because the plating efficiency of the negative control was only 50 %. The data has been taken as basis for the dose selection of these repeat experiments 090M95/ 3 and /2.

Mutagenicity testing:

Without S9 Activation: The sponsor reports a "slight, but statistically significant" increase in mutant frequency in cultures treated with 200 µg/ml of the tolcapone/Sinemet combination (Table 4.c). This statement is based on an approximate 1.6-fold increase in total colony number. Inspection of the data on the formation of small colonies, which presumably reflects a greater degree of chromosomal damage, reveals a 2.7-fold increase in mutant frequency. This is not indicated as statistically significant, but the magnitude of effect appears more dramatic than the marginal increase in total colony number.

Table 4.c : Experiment 090M95/7

Raw plate counts, viability, TK mutants and mutation frequency of mouse lymphoma *tk*$^+$/*tk*$^-$ cells after 3 h exposure to Tolcapone/Sinemet without metabolic activation.

Test article	Conc. µg/ml	Viability : Survivor I[1] EW	EW	PE (%)	RS (%)	Viability : Survivor II[1] EW	EW	PE (%)	TK Mutants[2] EW	EW	EW	EW	EW	MF[3] colony large	colony small	colony total
RPMI	0	36 29	33	68	100	32 30	31	71	74	70	72	73	72	201		
DMSO	0	33 30	32	70	100	32 31	32	70	80	80	76	72	77	180		
Tolcapone/Sinemet	40	34 36	35	63	93	29 23	26	62	73	74	78	72	74	157		
Tolcapone/Sinemet	40	39 33	36	61	90	35 26	31	72	79	76	80	79	79	140		
Tolcapone/Sinemet	70	57 56	57	33	49	28 38	33	67	75	79	71	79	76	176		
Tolcapone/Sinemet	70	44 58	51	40	59	31 28	30	74	72	71	75	69	72	200		
Tolcapone/Sinemet	120	69 66	68	22	32	29 31	30	73	73	75	78	72	75	174		
Tolcapone/Sinemet	120	64 65	65	25	37	32 35	34	66	71	75	71	75	73	205		
Tolcapone/Sinemet	200	81 82	82	10	15	37 40	39	57	74	69	74	66	71	264		
Tolcapone/Sinemet	200	89 83	86	7	10	41 35	38	58	80	79	82	76	79	165		
NQO	0.1	55 47	51	40	67	41 39	40	55	59	59	60	60	62	402		

[1] 1.6 cells seeded per well

[2] 2×10^5 cells seeded per well

[3] per 10^6 viable cells

continued on next page

Mu 12/95

Study No.090M95

Table 4.c : Experiment 090M95/7

Raw plate counts, viability, TK mutants and mutation frequency of mouse lymphoma *tk*$^+$/*tk*$^-$ cells after 3 h exposure to Tolcapone/Sinemet without metabolic activation.

Test article	Conc. µg/ml	Viability : Survivor I[1] EW	EW	PE (%)	RS (%)	Viability : Survivor II[2] EW	EW	PE (%)	TK Mutants[3] EW	EW	EW	EW	EW	MF[3] colony large	colony small	colony total
RPMI	0	36 29	33	68	100	32 30	31	71	74	70	72	73	72	201		
DMSO	0	33 30	32	70	100	32 31	32	70	80	80	76	72	77	160		
Tolcapone	100	59 66	63	27	40	38 30	34	66	85	73	74	80	78	160		
Tolcapone	100	62 57	60	30	44	35 29	32	69	67	79	66	73	71	217		
Sinemet	100	42 35	39	57	84	34 26	30	73	78	72	72	73	73	186		
Sinemet	100	39 51	45	47	69	29 22	26	63	73	79	75	78	76	139		
NQO	0.1	55 47	51	40	57	41 39	40	55	59	59	69	60	62	402		

[1] 1.6 cells seeded per well

[2] 2 x10^6 cells seeded per well

[3] per 10^6 viable cells

With S9 Activation: A concentration-related increase in mutant frequency was evident in cells treated with the combination of tolcapone and Sinemet in two different experiments (Tables 4b, 4d). The magnitude of increase was most evident in small colony formation. Cell viability at the concentration of 250 µg/ml was 26-36%. Experiments with the individual drugs indicated that the effect of the combination was due to tolcapone. The positive controls produced the expected results.

Table 4.b : Experiment 090M95/4

Raw plate counts, viability, TK mutants and mutation frequency of mouse lymphoma *tk*$^+$/*tk*$^-$ cells after 3 h exposure to Tolcapone/Sinemet with metabolic activation.

Mu 1295

Test article	Conc. µg/ml	Viability : Survivor I[1] EW	EW	PE (%)	RS (%)	Viability : Survivor II[2] EW	EW	PE (%)	TK Mutants[3] EW	EW	EW	EW	EW	MF[3] colony large	colony small	colony total
RPMI	0	24 38	31	71	100	24 30	27	79	76	80	74	68	75	160		
DMSO	0	23 25	24	67	100	32 24	28	77	76	77	73	81	77	147		
Tolcapone/Sinemet	12.50	31 20	26	63	117	27 31	29	75	79	74	69	84	80	122		
Tolcapone/Sinemet	25	24 29	27	60	113	30 26	28	77	84	73	79	79	79	129		
Tolcapone/Sinemet	50	33 29	31	71	100	20 26	23	89	76	76	82	80	79	113		
Tolcapone/Sinemet	100	46 46	46	46	65	21 24	23	91	82	80	78	82	81	97		
Tolcapone/Sinemet	200	67 72	70	20	28	32 29	31	72	77	76	78	79	78	149		
Tolcapone/Sinemet	300	88 89	89	5	7	33 43	38	58	71	76	79	77	78	205		
Sinemet	50	36 39	38	58	83	32 22	27	79	77	75	80	79	76	133		
Sinemet	150	29 28	28	78	110	24 27	26	83	75	76	71	69	73	167		
BP	2	45 39	42	52	60	30 37	34	66	61	59	65	57	61	351		

Study No.090M95

[1] 1.6 cells seeded per well

[2] 2 x10^6 cells seeded per well

[3] per 10^6 viable cells

Table 4.d : Experiment 090M95/6

Raw plate counts, viability, TK mutants and mutation frequency of mouse lymphoma *tk*$^{+}$/*tk*$^{-}$ cells after 3 h exposure to Tolcapone/Sinemet with metabolic activation.

Mu 12/95

Study No. 090M95

Test article	Conc. µg/ml	Viability : Survivor I[1] EW	EW	PE (%)	RS (%)	Viability : Survivor II[1] EW	EW	PE (%)	TK Mutants[2] EW	EW	EW	EW	EW	MF[3] colony large	colony small	colony total
RPMI	0	37 30	34	66	100	30 35	33	66	73	80	77	71	75	179		
DMSO	0	32 35	34	66	100	35 33	34	65	80	80	84	74	80	145		
Tolcapone/Sinemet	50	29 30	30	74	112	30 30	30	73	71	70	69	69	70	219		
Tolcapone/Sinemet	50	28 26	27	76	120	28 33	31	72	70	73	74	75	73	191		
Tolcapone/Sinemet	90	51 37	44	49	74	37 42	40	66	81	80	79	78	80	170		
Tolcapone/Sinemet	90	44 48	46	46	70	36 43	41	54	66	84	65	67	70	289		
Tolcapone/Sinemet	150	56 66	61	29	44	43 40	42	52	80	77	80	78	79	188		
Tolcapone/Sinemet	150	64 62	63	26	39	40 42	41	53	76	84	80	79	80	177		
Tolcapone/Sinemet	250	70 77	74	17	26	49 43	46	46	83	83	71	77	79	219		
Tolcapone/Sinemet	250	68 62	65	24	36	46 42	45	47	67	74	76	73	73	296		
BP	2	50 42	46	46	70	45 41	43	50	56	62	60	63	58	505		

Test article	Conc. µg/ml	Viability : Survivor I[1] EW	EW	PE (%)	RS (%)	Viability : Survivor II[1] EW	EW	PE (%)	TK Mutants[2] EW	EW	EW	EW	EW	MF[3] colony large	colony small	colony total
RPMI	0	37 30	34	66	100	30 35	33	66	73	80	77	71	75	179		
DMSO	0	32 35	34	66	100	35 33	34	65	80	80	84	74	80	145		
Tolcapone	125	59 63	61	28	42	42 36	39	56	77	71	69	79	74	231		
Tolcapone	125	66 73	70	20	30	44 42	43	50	76	71	71	70	73	280		
Sinemet	125	32 31	32	70	106	30 50	40	55	70	80	74	69	73	247		
Sinemet	125	33 25	29	75	114	34 42	38	58	81	86	71	76	74	226		
BP	2	50 42	46	46	70	45 41	43	50	56	62	60	63	58	505		

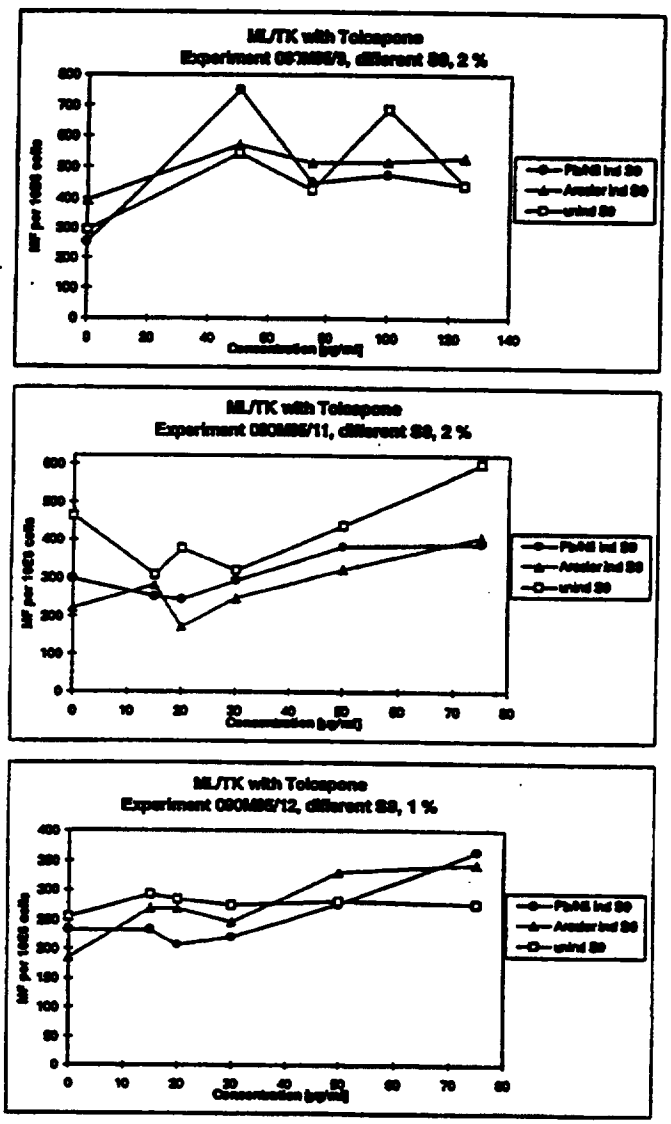
[1] 1.6 cells seeded per well

[2] 2 x10^{5} cells seeded per well

[3] per 10^{6} viable cells

The source of S9 fractions had no influence on mutant frequency as tolcapone had comparable effects with both types of induced and uninduced microsome fractions (Figure 3).

Figure 3: Mutant frequencies after 3 h exposure of Tolcapone with differently induced metabolic activation systems - Experiments 0908/95/9, /11 and /12



C.4.h. *In vivo* **Mouse Micronucleus Test**

Research Report #: B-153,599
Sponsor Volume: 52

Summary:

In this *in vivo* mutagenicity study, single doses of tolcapone (300 mg/kg, p.o.) did not significantly increase the number of micronucleated polychromatic erythrocytes in the femoral bone marrow of mice at 24-72 hrs after dosing. The positive control procarbazine produced the expected result.

No description of clinical signs of toxicity or lethality was provided, so the adequacy of the dosage levels could not be determined. In addition, the number of polychromatic erythrocytes scored for the presence of micronuclei was lower than that recommended in the 1994 OECD guidelines (1000 vs recommended 2000). Thus, the acceptability of this assay for assessing potential genotoxic effects of tolcapone is questionable.

Methods:

Dosage/Route: Tolcapone (Lot G PUL 493 089) was administered by gavage at doses of 150 and 300 mg/kg. Drug was prepared in SSV to deliver 10 ml/kg.

The high dose was selected on the basis of a preliminary toxicity study in which sublethal effects occurred at 312 mg/kg.

Positive Control: Procarbazine HCl, 50 mg/kg, in PBS (this agent is not on the OECD list of recommended positive controls).

Animals: Fu-Moro albino mice, 37-42 g
15 males and 15 females were used in the negative control and high dose groups. Five males and 5 females were used for the low dose group, and 5 males were used for the positive control group.

Note: Failure to comply with OECD guidelines stating that 3 dose levels, or a single dose level of 2000 mg/kg/day should be employed.

Sample Collection and Analysis:

Five animals per group were sacrificed at 24, 48 and 72 hr post-treatment (in groups that contained only 5 animals, all were sacrifiecd at 24 hrs). Femoral marrow smears were fixed on a slide (2 per animal) and stained with May-Grunwald-Giemsa. The number of polychromatic erythrocytes scored for the presence of micronuclei 1000 per animal.

Note: OECD guidelines state that 2000 PCEs should be scored.

Statistics

Comparisons were made by the Mann-Whitney U-test.

Results:

Single doses of 150 or 300 mg/kg tolcapone to male and female mice did not significantly increase the number of micronucleated polychromatic erythrocytes (MN-PCEs) relative to control levels at 24 hrs after treatement (Table 1a). The high test dose also did not increase the number of MN-PCEs at 48 or 72 hrs (Tables 1b,c). The ratio of polychromatic to normochromatic erythrocytes was similar in control and treated animals. The positive control procarbazine produced the expected increase in MN-PCEs (Table 2).

These data suggest that tolcapone is not genotoxic in the *in vivo* mouse micronucleus assay. Although the sponsor states that the high dose was selected based on observations in an acute toxicity study, no description of clinical signs of toxicity in the present study were provided. This raises the question of whether the doses used in this study were adequate. In addition, the number of scored PCEs was lower than the recommended number in the OECD guidelsines (2000/animal).

Table 1a : Micronucleus Test with Fuellinsdorf Albino Mice (SPF)
Treated with RO 40-7592/001. Mode of application: ORAL
Sampling time: 24 h

Single Dose mg/kg	Animal No.	Sex	NCE with MN No.	%	Ratio PCE/NCE	Median	PCE with MN No.	%	Median + Significance levels
0	111	m	2	0.23	1.13		2	0.20	
	112	m	2	0.18	0.88		2	0.20	
	113	m	2	0.19	0.93		5	0.50	
	114	m	5	0.47	0.93		2	0.20	
	115	m	0	0.00	1.17		7	0.70	
						1.06			0.20
	117	f	3	0.38	1.28		1	0.10	
	118	f	0	0.00	1.00		1	0.10	
	119	f	1	0.15	1.52		3	0.30	
	1110	f	1	0.10	0.98		3	0.30	
	1111	f	1	0.16	1.62		2	0.20	
150	411	m	4	0.35	0.89		5	0.50	
	412	m	0	0.00	1.22		5	0.50	
	413	m	1	0.10	1.03		6	0.60	
	414	m	3	0.21	0.71		0	0.00	
	415	m	0	0.00	1.25		4	0.40	
						0.98			0.40 n.s.
	417	f	1	0.08	0.85		1	0.10	
	418	f	3	0.26	0.87		4	0.40	
	419	f	2	0.27	1.37		4	0.40	
	4110	f	0	0.00	0.99		1	0.10	
	4111	f	2	0.20	0.98		0	0.00	
300	511	m	2	0.13	0.66		6	0.60	
	512	m	3	0.27	0.89		4	0.40	
	513	m	1	0.12	1.17		6	0.60	
	514	m	0	0.00	1.98		1	0.10	
	515	m	2	0.21	1.06		1	0.10	
						1.14			0.30 n.s.
	517	f	2	0.24	1.21		2	0.20	
	518	f	0	0.00	1.12		2	0.20	
	519	f	3	0.53	1.77		4	0.40	-
	5110	f	1	0.09	0.95		3	0.30	=
	5111	f	2	0.23	1.15		3	0.30	= =

Experiment Number: 62-M-89
No.of PCE scored per animal: 1000

n.s. = no significance
X for P =< 0.05 XX for P =< 0.01
Trend : (+) increasing / (-) decreasing

Single Dose mg/kg	Animal No.	Sex	NCE with MN No.	%	Ratio PCE/NCE	Median	PCE with MN No.	%	Median + Significance Levels
0	121	m	2	0.19	0.96		7	0.70	
	122	m	2	0.14	0.68		8	0.80	
	123	m	0	0.00	1.11		3	0.30	
	124	m	0	0.00	0.95		4	0.40	
	125	m	1	0.10	0.98		2	0.20	
						1.07			0.30
	127	f	0	0.00	1.07		3	0.30	
	128	f	1	0.13	1.28		1	0.10	
	129	f	2	0.28	1.38		2	0.20	
	1210	f	1	0.11	1.07		2	0.20	
	1211	f	3	0.44	1.45		3	0.30	
300	521	m	0	0.00	1.28		7	0.70	
	522	m	0	0.00	1.87		4	0.40	
	523	m	1	0.15	1.46		2	0.20	
	524	m	2	0.34	1.72		1	0.10	
	525	m	3	0.13	0.43		3	0.30	
						1.30			0.30 n.s.
	527	f	3	0.40	1.32		5	0.50	
	528	f	1	0.12	1.16		3	0.30	
	529	f	4	0.47	1.17		2	0.20	
	5210	f	1	0.13	1.25		3	0.30	
	5211	f	1	0.19	1.94		3	0.30	

Tab. 1b. 48hr

Single Dose mg/kg	Animal No.	Sex	NCE with MN No.	%	Ratio PCE/NCE	Median	PCE with MN No.	%	Median + Significance Levels
0	131	m	1	0.12	1.22		2	0.20	
	132	m	1	0.15	1.48		5	0.50	
	133	m	0	0.00	1.44		8	0.80	
	134	m	1	0.11	1.08		3	0.30	
	135	m	1	0.12	1.16		7	0.70	
						1.19			0.35
	137	f	1	0.14	1.40		4	0.40	
	138	f	4	0.29	0.72		4	0.40	
	139	f	1	0.14	1.40		2	0.20	
	1310	f	0	0.00	1.03		3	0.30	
	1311	f	1	0.11	1.13		1	0.10	
300	531	m	3	0.35	1.16		0	0.00	
	532	m	1	0.08	0.78		2	0.20	
	533	m	5	0.52	1.05		3	0.30	
	534	m	3	0.19	0.63		2	0.20	
	535	m	5	0.58	1.15		4	0.40	
						1.10			0.25 n.s.
	537	f	2	0.14	0.70		1	0.10	
	538	f	0	0.00	1.23		5	0.50	
	539	f	4	0.40	1.00		5	0.50	
	5310	f	0	0.00	1.38		3	0.30	
	5311	f	1	0.14	1.43		0	0.00	

Tab. 1c. 72hr

Single Dose mg/kg	Animal No.	Sex	NCE with MN No.	%	Ratio PCE/NCE	Median	PCE with MN No.	%	Median + Significance Levels
0	111	m	2	0.23	1.13		2	0.20	
	112	m	2	0.18	0.88		2	0.20	
	113	m	2	0.19	0.93		5	0.50	
	114	m	5	0.47	0.93		2	0.20	
	115	m	0	0.00	1.17		7	0.70	
						1.06			0.20
	117	f	3	0.38	1.28		1	0.10	
	118	f	0	0.00	1.00		1	0.10	
	119	f	1	0.15	1.52		3	0.30	
	1110	f	1	0.10	0.98		3	0.30	
	1111	f	1	0.16	1.62		2	0.20	
50	611	m	5	0.35	0.70		36	3.60	
	612	m	3	0.22	0.74		49	4.90	
	613	m	2	0.18	0.91		68	6.80	
	614	m	1	0.12	1.15	0.91	58	5.80	4.90 xx(+)
	615	m	2	0.21	1.03		39	3.90	

Tab 2. positive control

Experiment Number: 62-M-89

No.of PCE scored per animal: 1000

n.s. = no significance

x for P =< 0.05 xx for P =< 0.01

Trend : (+) increasing / (-) decreasing

C.4.i. *In vivo* Mouse Micronucleus Test with Tolcapone in Combination with Sinemet

Research Report #: B-164,908
Sponsor Volume: 52

Summary:

Single oral doses of up to 300 mg/kg tolcapone and Sinemet, alone and in combination (total dose = 600 mg/kg), were evaluated for mutagenic effects *in vivo* in the mouse micronucleus test. No significant increases in the number of micronucleated polychromatic erythrocytes in the femoral bone marrow of mice at 24 and 48 hrs after dosing. The positive control procarbazine produced the expected result. Thus, tolcapone and Sinemet, alone and in combination, were not mutagenic in the *in vivo* mouse micronucleus assay under the conditions employed in this study.

Since tolcapone appears to be species (rat)-specific with respect to renal tumor formation, the rat may have been a more appropriate model for this *in vivo* micronucleus drug combination study. The finding that rat S9 activation tended to increase potential mutagenic effects of tolcapone in the ML/TK assay suggests the possibility that a species-specific metabolite may be involved in the genotoxic mechanism. The genotoxic activity of a rat-specific metabolite obviously would not be detected in an *in vivo* mouse assay.

Methods:

Dosage Groups: Tolcapone (Lot G PUL 40802440) and Sinemet were prepared in SSV to be delivered by gavage according to the following scheme:

Test chemical	Dose mg/kg	Volume ml/kg	Number of mice treated male	Number of mice treated female	Number of mice evaluated for MN male	Number of mice evaluated for MN female
Solvent control: SSV	0	10	10	10	10	10
Tolcapone (Ro 40-7592/001)	150	10	5	5	5	5
"	300	10	11	12	10	10
Sinemet (Ro 20-3828/000, Ro 05-4759/000, 1:4)	300	10	10	10	10	10
Tolcapone/Sinemet (Ro 40-7592/001, Ro 20-3828, Ro 05-4759/000, 5:1:4)	150	10	5	5	5	5
"	300	10	5	5	5	5
"	600	10	12	11	10	10
Positive control: Procarbazine-HCl (Ro 04-6467/001)	50	10	5		5	

The high dose of the combination was appropriately selected as an MTD on the basis of a preliminary toxicity study in which significant lethality occurred at the next highest dosage level (800 mg/kg).

Procarbazine is not listed in OECD guidelines as ~~an acceptable~~ recommended positive control.

Animals: Fu-Moro albino mice; mean weight of males: 42.3g, females: 32.8g

Five animals per group were sacrificed at 24 and 48 hr post-treatment. In groups that contained only 5 animals, all were sacrificed at 24 hrs. Femoral marrow smears were fixed on a slide (2 per animal) and stained with May-Grunwald-Giemsa. The number of polychromatic erythrocytes scored for the presence of micronuclei was 2000 per animal.

Statistics:

Evaluations were made by the Mann-Whitney U-test.

Results:

The combination of tolcapone and Sinemet (Table 1), or single doses of tolcapone (Table 2) and Sinemet (Table 3) alone did not significantly increase the number of micronucleated polychromatic erythrocytes (MN-PCEs) relative to control levels in male and female mice at either interval (24, 48 hrs) after treatment. The ratio of polychromatic to normochromatic erythrocytes was similar in control and treated animals. The positive control procarbazine produced the expected increase in MN-PCEs. These data suggest that tolcapone, alone or in combination with Sinemet, is not genotoxic in the *in vivo* mouse micronucleus assay under the conditions of the present study.

Tab. 1 - Tolcapone + Sinemet

Table 1a : Micronucleus Test with Fuellinsdorf Albino Mice (SPF)

Treated with TOLC./SIN. . Mode of application: ORAL

Sampling time: 24 h

Single Dose mg/kg	Animal No.	Sex	NCE with MN No.	%	Ratio PCE/NCE	Median	PCE with MN No.	%	Median + Significance Levels
0	111	m	3	0.10	0.67	1.30	3	0.15	0.10
	112	m	5	0.19	1.24		1	0.05	
	113	m	5	0.08	0.33		4	0.20	
	114	m	0	0.00	1.24		2	0.10	
	115	m	1	0.09	1.82		5	0.25	
	116	f	2	0.12	1.23		2	0.10	
	117	f	0	0.00	1.84		5	0.25	
	118	f	3	0.20	1.34		2	0.10	
	119	f	0	0.00	1.68		0	0.00	
	1110	f	2	0.11	1.07		1	0.05	
150	711	m	3	0.21	1.42	1.31	5	0.25	0.15 n.s.
	712	m	2	0.16	1.65		5	0.25	
	713	m	1	0.04	0.88		3	0.15	
	714	m	2	0.15	1.53		4	0.20	
	715	m	0	0.00	0.98		2	0.10	
	716	f	1	0.13	2.68		2	0.10	
	717	f	2	0.11	1.07		3	0.15	
	718	f	3	0.26	1.73		3	0.15	
	719	f	0	0.00	1.07		4	0.20	
	7110	f	1	0.06	1.19		2	0.10	
300	611	m	2	0.07	0.74	1.32	2	0.10	0.13 n.s.
	612	m	2	0.10	1.00		5	0.25	
	613	m	4	0.19	0.97		5	0.25	
	614	m	2	0.12	1.24		3	0.15	
	615	m	2	0.17	1.75		3	0.15	
	616	f	0	0.00	1.42		1	0.05	
	617	f	1	0.05	1.05		3	0.15	
	618	f	2	0.18	1.79		2	0.10	
	619	f	1	0.07	1.40		1	0.05	
	6110	f	1	0.05	1.84		0	0.00	
600	511	m	2	0.12	1.21	1.21	7	0.35	0.13 n.s.
	512	m	2	0.12	1.25		6	0.30	
	513	m	3	0.16	1.09		4	0.20	
	514	m	1	0.03	0.63		0	0.00	
	515	m	6	0.22	0.73		3	0.15	
	516	f	2	0.13	1.28		2	0.10	
	517	f	2	0.13	1.34		2	0.10	
	518	f	4	0.24	1.21		3	0.15	
	519	f	1	0.06	1.27		1	0.05	
	5110	f	1	0.04	0.72		1	0.05	

Experiment Number: 079M95/2

No.of PCE scored per animal: 2000

n.s. = no significance

* for P =< 0.05 ** for P =< 0.01

Trend : (+) increasing / (-) decreasing

Table 1d : Micronucleus Test with Fuellinsdorf Albino Mice (SPF)

Treated with TOLC./SIN. . Mode of application: ORAL

Sampling time: 48 h

Single Dose mg/kg	Animal No.	Sex	NCE with MN No.	%	Ratio PCE/NCE	Median	PCE with MN No.	%	Median + Significance Levels
0	121	m	1	0.06	1.11	1.16	2	0.10	0.10
	122	m	1	0.06	1.22		5	0.25	
	123	m	1	0.14	2.73		3	0.15	
	124	m	1	0.06	1.16		2	0.10	
	125	m	4	0.15	0.77		6	0.30	
	126	f	1	0.06	1.16		2	0.10	
	127	f	0	0.00	1.72		0	0.00	
	128	f	0	0.00	0.98		2	0.10	
	129	f	2	0.11	1.06		4	0.20	
	1210	f	2	0.14	1.43		1	0.05	
600	521	m	2	0.19	1.94	1.34	4	0.20	0.13 n.s.
	522	m	3	0.28	1.30		3	0.15	
	523	m	3	0.09	0.60		3	0.15	
	524	m	4	0.23	1.13		4	0.20	
	525	m	0	0.00	1.28		1	0.05	
	526	f	0	0.00	1.39		3	0.15	
	527	f	1	0.06	1.17		0	0.00	
	528	f	1	0.07	1.37		1	0.05	
	529	f	0	0.00	1.72		1	0.05	
	5210	f	1	0.08	1.57		2	0.10	

Experiment Number: 079M95/2

No.of PCE scored per animal: 2000

n.s. = no significance

* for P =< 0.05 ** for P =< 0.01

Trend : (+) increasing / (-) decreasing

Tab.2 - Tolcapone

Table 2 : Micronucleus Test with Fuellinsdorf Albino Mice (SPF)
Treated with TOLCAPONE . Mode of application: ORAL
Sampling time: 24 h

Single Dose mg/kg	Animal No.	Sex	NCE with MN No.	%	Ratio PCE/NCE	Median	PCE with MN No.	%	Median + Significance Levels
0	111	m	3	0.10	0.67	1.30	3	0.15	0.10
	112	m	3	0.19	1.24		1	0.05	
	113	m	5	0.08	0.33		4	0.20	
	114	m	0	0.00	1.54		2	0.10	
	115	m	1	0.09	1.82		5	0.25	
	116	f	2	0.12	1.23		2	0.10	
	117	f	0	0.00	1.86		7	0.35	
	118	f	3	0.20	1.36		2	0.10	
	119	f	0	0.00	1.48		0	0.00	
	1110	f	2	0.11	1.07		1	0.05	
150	311	m	1	0.10	1.94	0.92	5	0.25	0.15 n.s.
	312	m	1	0.06	1.13		3	0.15	
	313	m	1	0.06	0.43		4	0.20	
	314	m	0	0.00	0.86		5	0.25	
	315	m	1	0.05	0.69		2	0.10	
	316	f	0	0.00	1.04		2	0.10	
	317	f	0	0.00	0.76		4	0.20	
	318	f	3	0.15	0.97		2	0.10	
	319	f	1	0.04	0.88		1	0.05	
	3110	f	1	0.07	1.41		3	0.15	
300	211	m	5	0.14	0.90	0.82	4	0.20	0.10 n.s.
	212	m	3	0.11	0.73		1	0.05	
	213	m	6	0.25	0.83		2	0.10	
	214	m	2	0.08	0.80		1	0.05	
	215	m	2	0.16	1.62		2	0.10	
	216	f	0	0.00	0.59		2	0.10	
	217	f	2	0.13	1.30		3	0.15	
	218	f	7	0.18	0.52		4	0.20	
	219	f	2	0.07	0.64		1	0.05	
	2110	f	2	0.09	0.89		1	0.05	

Experiment Number: 079N19S/2
No.of PCE scored per animal: 2000

n.s. = no significance
* for P =< 0.05 ** for P =< 0.01
Trend : (+) increasing / (-) decreasing

Table 2 : Micronucleus Test with Fuellinsdorf Albino Mice (SPF)
Treated with TOLCAPONE . Mode of application: ORAL
Sampling time: 48 h

Single Dose mg/kg	Animal No.	Sex	NCE with MN No.	%	Ratio PCE/NCE	Median	PCE with MN No.	%	Median + Significance Levels
0	121	m	1	0.06	1.11	1.16	2	0.10	0.10
	122	m	1	0.06	1.22		5	0.25	
	123	m	1	0.14	2.73		3	0.15	
	124	m	1	0.06	1.16		2	0.10	
	125	m	4	0.15	0.77		6	0.30	
	126	f	1	0.06	1.16		2	0.10	
	127	f	0	0.00	1.72		0	0.00	
	128	f	0	0.00	0.98		2	0.10	
	129	f	2	0.11	1.06		4	0.20	
	1210	f	2	0.14	1.43		1	0.05	
300	221	m	3	0.20	1.31	1.22	4	0.20	0.13 n.s.
	222	m	5	0.35	1.40		10	0.50	
	223	m	2	0.16	1.62		4	0.20	
	224	m	2	0.09	0.92		3	0.15	
	225	m	1	0.03	0.61		1	0.05	
	226	f	1	0.06	1.11		0	0.00	
	227	f	0	0.00	1.98		3	0.15	
	228	f	2	0.11	1.12		2	0.10	
	229	f	2	0.13	1.35		2	0.10	
	2210	f	2	0.10	1.02		1	0.05	

Experiment Number: 079N19S/2
No.of PCE scored per animal: 2000

n.s. = no significance
* for P =< 0.05 ** for P =< 0.01
Trend : (+) increasing / (-) decreasing

Tab. 3

Table 3 : Micronucleus Test with Fuellinsdorf Albino Mice (SPF)
Treated with SINEMET . Mode of application: ORAL
Sampling time: 24 h

Single Dose mg/kg	Animal No.	Sex	NCE with MN No.	%	Ratio PCE/NCE	Median	PCE with MN No.	%	Median + Significance Levels
0	111	m	3	0.10	0.67		3	0.15	
	112	m	3	0.19	1.24		1	0.05	
	113	m	5	0.08	0.33		4	0.20	
	114	m	0	0.00	1.54		2	0.10	
	115	m	1	0.09	1.82	1.30	5	0.25	0.10
	116	f	2	0.12	1.23		2	0.10	
	117	f	0	0.00	1.56		7	0.35	
	118	f	3	0.20	1.36		2	0.10	
	119	f	0	0.00	1.68		0	0.00	
	1110	f	2	0.11	1.07		1	0.05	
300	411	m	1	0.04	0.88		5	0.25	
	412	m	0	0.00	1.60		5	0.25	
	413	m	2	0.11	1.12		4	0.20	
	414	m	1	0.03	0.63		2	0.10	
	415	m	1	0.04	0.78	0.98	2	0.10	0.15 n.s.
	416	f	0	0.00	1.51		3	0.15	
	417	f	2	0.07	0.68		3	0.15	
	418	f	3	0.19	1.24		2	0.10	
	419	f	0	0.00	1.02		0	0.00	
	4110	f	2	0.09	0.93		1	0.05	

Sampling time: 48 h

Single Dose mg/kg	Animal No.	Sex	NCE with MN No.	%	Ratio PCE/NCE	Median	PCE with MN No.	%	Median + Significance Levels
0	121	m	1	0.06	1.11		2	0.10	
	122	m	1	0.04	1.22		5	0.25	
	123	m	1	0.14	2.73		3	0.15	
	124	m	1	0.06	1.16		2	0.10	
	125	m	4	0.15	0.77	1.16	6	0.30	0.10
	126	f	1	0.06	1.16		2	0.10	
	127	f	0	0.00	1.72		0	0.00	
	128	f	0	0.00	0.98		2	0.10	
	129	f	2	0.11	1.06		4	0.20	
	1210	f	2	0.14	1.43		1	0.05	
300	421	m	4	0.22	1.11		1	0.05	
	422	m	1	0.04	1.17		4	0.20	
	423	m	1	0.06	1.18		3	0.15	
	424	m	1	0.07	1.36		5	0.25	
	425	m	0	0.00	0.89	1.35	1	0.05	0.15 n.s.
	426	f	3	0.24	1.60		2	0.10	
	427	f	1	0.07	1.34		1	0.05	
	428	f	0	0.00	1.79		4	0.20	
	429	f	0	0.00	1.66		3	0.15	
	4210	f	3	0.22	1.48		3	0.15	

Experiment Number: 079M95/2
No.of PCE scored per animal: 2000

n.s. = no significance
* for P =< 0.05 ** for P =< 0.01
Trend : (+) increasing / (-) decreasing

Table 4 : Micronucleus Test with Fuellinsdorf Albino Mice (SPF)
Treated with RO 04-6467/001. Mode of application: ORAL
Sampling time: 24 h

positive Control

Single Dose mg/kg	Animal No.	Sex	NCE with MN No.	%	Ratio PCE/NCE	Median	PCE with MN No.	%	Median + Significance Levels
0	111	m	3	0.10	0.67		3	0.15	
	112	m	3	0.19	1.24		1	0.05	
	113	m	5	0.08	0.33		4	0.20	
	114	m	0	0.00	1.54		2	0.10	
	115	m	1	0.09	1.82	1.30	5	0.25	0.10
	116	f	2	0.12	1.23		2	0.10	
	117	f	0	0.00	1.56		7	0.35	
	118	f	3	0.20	1.36		2	0.10	
	119	f	0	0.00	1.68		0	0.00	
	1110	f	2	0.11	1.07		1	0.05	
50	811	m	1	0.03	0.63		44	2.20	
	812	m	2	0.11	1.12		39	1.95	
	813	m	10	0.42	0.84	0.87	43	2.15	2.15 **(+)
	814	m	4	0.17	0.87		54	2.70	
	815	m	3	0.14	0.96		37	1.85	

Experiment Number: 079M95/2
No.of PCE scored per animal: 2000

n.s. = no significance
* for P =< 0.05 ** for P =< 0.01
Trend : (+) increasing / (-) decreasing

C.6. Carcinogenicity

C.6.a. Mouse Carcinogenicity Study (95 weeks - male; 80 weeks - female)

GLP	Research Report: B-161,832	Sponsor Volumes:	52-58
Conducted by :	Hoffmann-LaRoche Ltd. CH-4002 Basel Switzerland		

Summary:

Tolcapone was administered in the diet at doses of 100, 300, 800 mg/kg/day to Hanlbm:NMRI mice (50/sex/dose group, 100/sex/control) for two years. An additional 20/sex/dose were used for toxicokinetic evaluations at weeks 6, 52, and 79. Relatively few non-neoplastic and no neoplastic lesions were associated with tolcapone administration.

According to the sponsor's analysis, body weight development was not impaired by drug treatment. An alternative means of analysis suggested a decrease in body weight gain in MDF and HDF (17-30%). However, final body weights did not differ among treatment groups.

A relatively high rate of mortality occurred over the course of the study. A tendency for increased mortality in treated females was noted, but this was not significant. The study was terminated when approximately 50% of animals in one of the treatment groups died. This was week 80 in females and week 95 in males.

The primary target organs with non-neoplastic lesion apparently due to tolcapone were the forestomach and liver. In the stomach, epithelial hyperplasia (MD and HD, both sexes) and inflammation of the forestomach (MDM, HDM) and cuticular ridge (MDM, HDM, HDF) were attributed to focal irritation by the drug. The relevance of these changes to humans, in which this organ is absent, is questionable. Liver changes were hepatocellular hypertrophy (MDM, HDM, HDF), granulocytosis (MDM, HDM, MDF), single cell necrosis (HDM), Kupffer cell proliferation (MDF, HDF, HDM), abscesses (HDM), and lymphoid cell infiltration (HDM). Most of these changes occurred at a relatively high rate in control animals, but the increased incidence in treated animals is considered drug-related. Abscesses were considered incidental. No pathogenic mechanism for these changes was established. The incidence of ovarian interstitial cell hyperplasia also appeared elevated in HDF, but this was not indicated as statistically significant and was not discussed in the text of the Pathology report.

Increases in plasma exposures to tolcapone were slightly less than dose-proportional. No accumulation was evident. Relative to plasma exposures in humans receiving the projected maintenance dose of 200 mg, t.i.d. (AUC_{0-24} = 80 µg.hr/ml), exposures in mice were:

LD:	0.5 - 1.0	times the human exposure
MD:	1.2 - 2.2	"
HD:	2.4 - 6.0	"

Methods:

Dosages: 100, 300, 800 mg/kg/day tolcapone

The high dose was selected to be close to the MTD (based on mortality in pilot studies) but cause minimal reductions in body weight gain.

Route of Administration: Drug-in-diet

Species/Strain/Number: Mouse (Hanlbm:NMRI, outbred SPF); 25-30 g (4-6 weeks).

Group	Ro 40-7592/001 (mg/kg/day)	Males, with animal #	Females, with animal #
A	control 1	50 (93'1612-1661)	50 (93'1362-1411)
B	100	50 (1662-1711)	50 (1412-1461)
C	300	50 (1712-1761)	50 (1462-1511)
D	800	50 (1762-1811)	50 (1512-1561)
E	control 2	50 (1812-1861)	50 (1562-1611)

Additional 20 males and 20 females were used in groups B, C and D for toxicokinetic investigations (protocol 162P93K); samples for profiles were taken in about week 6, months 12 and 18. These animals were immediately discarded following blood sampling.

Plasma samples were collected at 0700, 1500, 2300 hrs from 2M and 2F per treatment group; separate animals for each time point.

Statistics

Dose-effect relationships on body weight and hematology parameters were analyzed by procedures based on ranks (Jonckheere test, Mann-Whitney U-test). the following values were calculated and analyzed to assess drug effects on body weight:

1. total weight gain
2. growth rate as determined by a weighted sum of body weight gains

Statistical evaluation of neoplastic and non-neoplastic lesions was according to Peto et al. (1980) using the trend test with respect to dose. One-tailed probability levels for significant findings were 0.05 for rare neoplasms and 0.01 for common neoplasms. The occurrence of a neoplastic lesion was only regarded as significant if the incidence exceeded 5% in at least one sex/dose.

Results:

Mortality:

Sponsor Table a Tex

The following number of animals were found dead or killed in a moribund condition (autopsy in week 80 for females and week 95 for males; groups of 50 animals/sex, data from PATHDATA):

	control 1	control 2	100 mg/kg/d	300 mg/kg/d	800 mg/kg/d
males	18	24	27	24	24
females	21	20	29	24	30

As can be seen from this table, in the males the survival rate of less than 25 survivors occurred first in the low dose group, consequently all remaining males were necropsied. Overall, there was no obvious treatment-related or group-related trend as to the mortalities.

The sponsor's statistical analysis did not reveal a positive trend between dose and mortality, although there appeared to be a tendency for a higher incidence of mortality in treated females.

The study was terminated when a mortality incidence of 50% in any of the drug treatment groups was encountered. This occurred during week 80 in females and week 95 in females.

Body Weight:

The effect of tolcapone on body weight development is shown in Sponsor Figure 1.

According to the sponsor's text, only inconsistent retardations in body weight development, which never exceeded 10%, occurred over the course of the study. This conclusion is not consistent with the summary tables of statistical analyses prepared by the sponsor, or with a simple review of mean body weight differences at the beginning of the study and at study termination.

According to the sponsor's analysis, which **appears** to only include data from animals that survived the study, body weight gain was reduced by 16% in HDM, 15% in MDF and 29% in HDF:

STUDY : 162P93
MALE BODYWEIGHTS,STATISTICS in [g] from DAY 1 to 652

GROUP NO.ANIMAL		GROWTH-RATE	TOT.WEIGHT DIFFERENCE
A 32	MEAN STD.	39.48 8.86	13.48 5.16
B 23	MEAN STD.	33.49 * 9.54	14.11 4.24
C 26	MEAN STD.	29.66 ** 9.43	15.08 2.74
D 26	MEAN STD.	23.90 ** 9.66	11.49 4.34
E 26	MEAN STD.	34.17 ** 6.30	13.93 3.85

EXCLUDED : DAY 442, 470, 630, 631, 632, 633, 636, 637, 639, 640, 644

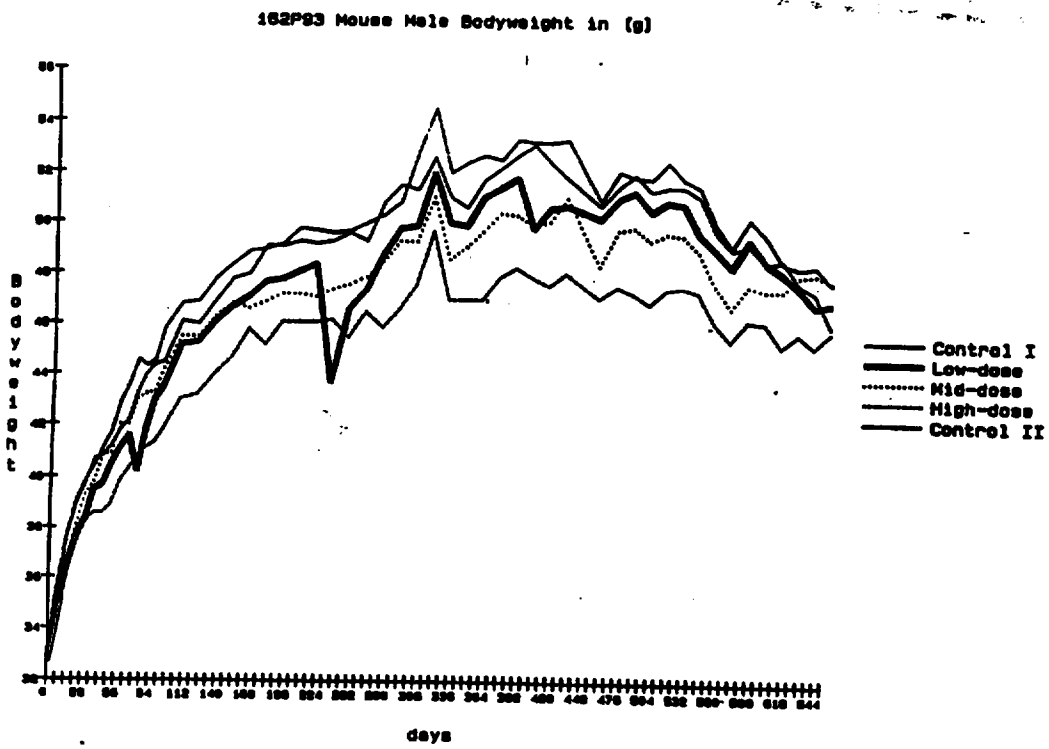
STUDY : 162P93
FEMALE BODYWEIGHTS,STATISTICS in [g] from DAY 0 to 553

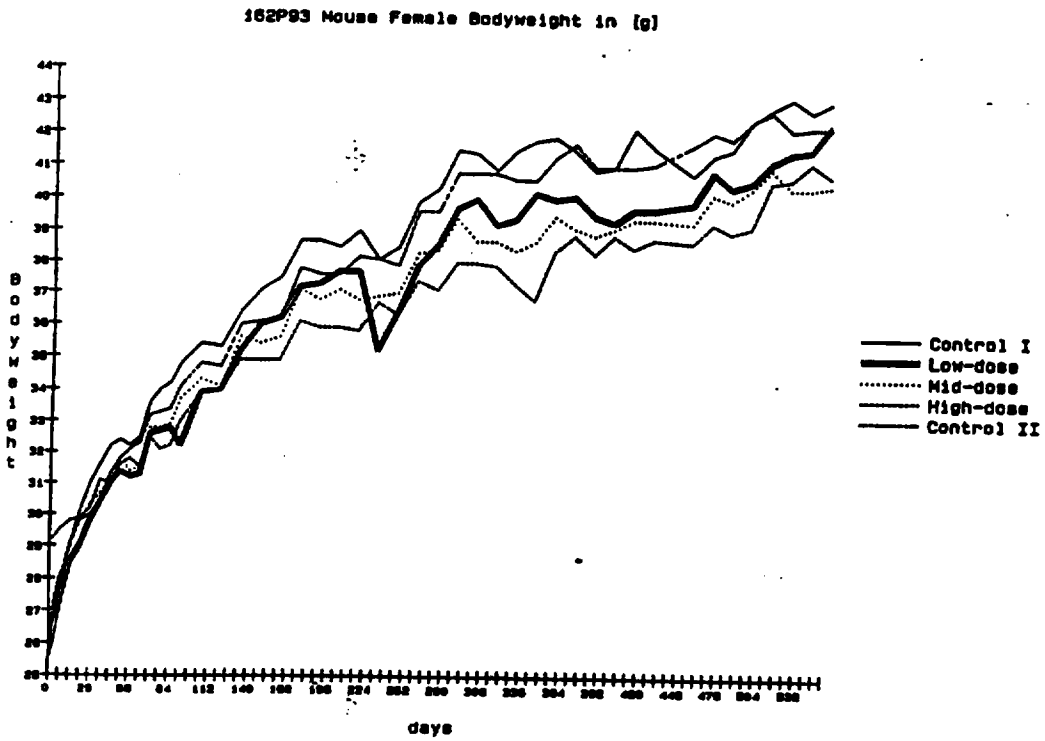
GROUP NO.ANIMAL		GROWTH-RATE	TOT.WEIGHT DIFFERENCE
A 31	MEAN STD.	21.89 6.11	16.72 4.17
B 22	MEAN STD.	19.93 9.28	16.45 3.72
C 26	MEAN STD.	16.30 ** 7.22	14.01 ** 4.03
D 21	MEAN STD.	8.63 ** 5.78	11.69 ** 4.85
E 30	MEAN STD.	16.47 ** 7.29	16.19 * 5.68

EXCLUDED : DAY 441, 469,

Jonckheere-Test : ** P <= 1%
* 1% < P <= 5%

U-Test : ## P <= 1%
1% < P <= 5%





Simply comparing group averages at study onset and termination produced similar differences relative to the controls in females, but only a 7% reduction in body weights in HDM:

MALES

Group	wgt - day 1	wgt - term.	diff	% control
C1	33.4	46.1	12.7	-
C2	33.8	47.8	14.0	-
100 mg/kg	32.7	47.0	14.3	107.1
300 "	33.5	47.9	14.4	107.8
800 "	33.5	45.9	12.4	92.9

FEMALES

Group	wgt - day 1	wgt - term.	diff	% control
C1	26.2	43.1	16.9	-
C2	26.4	42.3	15.9	-
100 mg/kg	25.6	42.4	16.8	102.4
300 "	26.9	40.5	13.6	82.9
800 "	29.1	40.8	11.7	71.3

Food Intake:

Measurements were taken weekly and appeared similar in all groups except for the high dose animals which tended to spill excessively (this may have led to a 10-20% reduction in drug intake at the high dose level).

Hematology: (at death or sacrifice)

Statistically significant group mean variations in surviving animals at termination:

↓ RBC (slight) - HDM
↑ MCH (slight) - HDM
↓ WBC - MDM
↑ platelets - HDM

These variations are not considered toxicologically significant.

Non-Neoplastic Lesions

Lesion			Group Incidence Rate (%) C1	C2	100	300	800
forestomach	epithelial hyperplasia	M	2	8	6	28*	78*
		F	4	10	10	16*	42*
	inflammation/forestomach	M	2	0	10	11*	38*
		F	2	4	4	10	2
	inflammation/cuticular ridge	M	0	2	0	11*	30*
		F	2	2	0	4	34*
liver	hepatocellular hypertrophy	M	16	49	40	78*	71*
		F	26	22	31	36	52*
	granulocytosis	M	2	12	12	16*	41*
		F	2	0	8	12*	0
	single cell necrosis	M	28	41	33	37	69*
		F	36	50	41	50	53
	Kupffer cell proliferation	M	8	33	29	16	45*
		F	30	33	45	54*	53*
	lymphoid cell infiltration	M	24	45	35	31	59*
		F	32	24	48	37	24
	abscesses	M	2	4	8	2	20
ovary	interstitial cell hyperplasia		4	0	2	2	19

*p < 0.05 by Cochran's Trend Test. Lesions were described as slight to moderate.

Interstitial cell hyperplasia was not indicated as statistically significant, although the incidence appears to be clearly higher in control animals.

Neoplastic lesions

No treatment-related neoplastic lesions were identified. In instances where tumors were identified only in drug-treated animals, the incidence rate did not exceed 5%. Significant p-values (<0.05) were obtained for the following tumors:

Males:

cecum adenocarcinoma:	2 HDM
hepatocellular carcinoma:	1 HDM
adrenal gland a-cell adenoma:	1 HDM
hemolymphoreticular histiocytic sarcoma:	1 LDM, 1HDM

Females:

uterine myxoma:	1 HDF

The original Pathology Summary tables can be found on subsequent pages.

Plasma Concentrations:

Plasma levels of tolcapone and its 3-O-methyl metabolite were determined at 0700, 1500, and 2300 hrs during weeks 6, 52 and 79. Increases in AUC for TASMAR were approximately dose-proportional. Drug accumulation was not evident. Very low levels of the 3-O-methyl metabolite were detected, and amounts were relatively similar in all treatment groups.

Table A : Pharmacokinetic parameters of Ro 40-7592

	C_{max} . (µg/ml)					
	Male mice			Female mice		
Dosage group	week 6	week 52	week 79	week 6	week 52	week 79
B	2.97	2.79	2.83	3.40	5.38	4.81
C	9.11	9.68	4.41	6.92	6.21	7.47
D	11.9	21.3	15.3	26.1	24.3	25.0

	AUC_{0-24} (h.µg/ml) *					
	Male mice			Female mice		
Dosage group	week 6	week 52	week 79	week 6	week 52	week 79
B	42.7	55.9	53.4	68.0	97.8	90.2
C	136	180	101	134	102	157
D	189	397	289	402	483	345

Table B : Pharmacokinetic parameters of Ro 40-7591

	C_{max} (µg/ml)					
	Male mice			Female mice		
Dosage group	week 6	week 52	week 79	week 6	week 52	week 79
B	0.606	0.390	0.218	0.505	0.270	0.167
C	0.582	0.317	0.241	0.469	0.180	0.165
D	0.571	0.295	0.214	0.467	0.197	0.182

	AUC_{0-24} (h.µg/ml) *					
	Male mice			Female mice		
Dosage group	week 6	week 52	week 79	week 6	week 52	week 79
B	8.48	7.34	4.33	8.79	4.34	3.04
C	10.0	6.32	3.71	8.39	3.55	2.79
D	9.00	4.78	3.45	8.11	4.06	3.14

Dosage group B : 100 mg/kg Ro 40-7592
Dosage group C : 300 mg/kg Ro 40-7592
Dosage group D : 800 mg/kg Ro 40-7592

AUC_{0-24} : 7.00 a.m. similar on 2 consecutive days .

TEST ARTICLE : BO 40-7592/001
TEST SYSTEM : MOUSE, 20 MONTHS, FEED-ADMIX
SPONSOR : HOFFMANN-LA ROCHE LTD
PATHOL. NO.: 96002 BJC
DATE : 12-MAR-96
PathData System V3.7

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

ORGAN/FINDING	DOSE GROUP	A		B		C		D		E	
	SEX	M	F	M	F	M	F	M	F	M	F
	NO. ANIMALS	50	50	50	50	50	50	50	50	50	50
BRAIN	No.Examined	49	50	50	49	48	50	49	50	48	48
- MENINGIOMA		1	-	-	-	-	-	-	-	-	-
HEART	No.Examined	50	50	50	50	50	50	50	50	49	49
- CONTACT METASTASIS		-	-	-	-	1	-	-	-	-	-
- METASTATIC SARCOMA		-	-	-	-	-	-	-	-	1	-
LUNGS	No.Examined	50	50	50	49	50	50	49	50	50	49
- BRONCHIOLAR-ALVEOLAR TUMOR		27	14	30	10	25	11	21	12	25	15
- METASTATIC CARCINOMA		-	-	1	-	1	-	-	-	-	-
- METASTATIC SARCOMA		-	-	-	2	1	-	-	1	2	-
STOMACH	No.Examined	48	50	49	48	47	50	49	50	48	49
- SQUAMOUS CELL CARCINOMA		-	-	-	-	-	-	-	-	2	-
- POLYP		-	-	-	-	-	-	-	1	-	-
DUODENUM	No.Examined	48	50	49	48	48	50	49	50	48	49
- POLYP		-	-	-	-	1	-	-	-	-	-
CECUM	No.Examined	48	50	49	46	48	49	49	50	49	49
- ADENOMA		-	-	-	-	-	-	-	-	1	-
- ADENOCARCINOMA		-	-	-	-	-	-	2	-	-	-
COLON	No.Examined	48	50	49	47	48	49	49	50	49	49
- POLYP		1	1	-	-	1	-	2	-	-	-
RECTUM	No.Examined	49	50	49	49	48	48	49	50	49	49
- POLYP		-	-	-	1	-	-	1	-	-	2
LIVER	No.Examined	50	50	46	49	49	50	49	49	49	49
- HEPATOCELLULAR ADENOMA		-	-	-	1	2	-	-	1	-	-
- HEPATOCELLULAR CARCINOMA		-	-	-	-	-	-	1	-	-	-
- METASTATIC CARCINOMA		-	-	2	-	-	-	-	-	1	-
- METASTATIC SARCOMA		-	-	-	-	-	-	-	1	-	-
- MESOTHELIOMA		-	-	1	1	-	-	2	1	1	-
- HEMANGIOSARCOMA		-	-	-	2	-	-	-	-	-	-

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NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

ORGAN/FINDING	DOSE GROUP	A		B		C		D		E	
	SEX	M	F	M	F	M	F	M	F	M	F
	NO. ANIMALS	50	50	50	50	50	50	50	50	50	50
ADRENAL GLANDS	No.Examined	49	49	49	50	49	49	48	50	49	49
- MEDULLARY TUMOR		-	-	1	-	1	-	2	-	2	-
- CORTICAL ADENOMA		2	-	-	2	-	-	1	-	3	-
- A-CELL ADENOMA		-	-	-	1	-	1	1	-	-	1
- BASALIOMAS		-	-	-	-	-	-	-	-	1	-
HEMOLYMPHORET. SYS.	No.Examined	50	50	50	50	50	50	50	50	50	50
- MALIGNANT LYMPHOMA		11	26	8	23	7	22	14	22	19	19
- HISTIOCYTIC SARCOMA		-	-	1	1	-	-	2	1	-	-
- MALIGNANT HISTIOCYTOSIS		-	-	-	-	-	-	-	-	1	-
BONE MARROW	No.Examined	50	50	50	49	50	50	50	50	50	50
- METASTATIC SARCOMA		-	-	-	-	1	-	-	-	-	-
THYMUS	No.Examined	49	46	49	46	49	50	50	49	49	49
- THYMOMA		-	-	-	-	1	-	-	-	-	-
- CARCINOSARCOMA		-	-	1	-	1	-	-	-	-	-
LYMPH NODES	No.Examined	49	49	49	48	47	49	49	50	47	49
- METASTATIC CARCINOMA		2	-	-	-	-	-	-	-	-	-
- METASTATIC SARCOMA		1	-	-	-	-	-	-	-	-	-
HARDERIAN GLANDS	No.Examined	50	50	49	49	50	50	50	50	50	49
- ADENOMA		2	1	2	1	3	-	-	1	1	1
- ADENOCARCINOMA		-	-	1	-	-	-	-	-	-	-
MAMMARY GLAND AREA	No.Examined	50	50	50	50	50	50	50	50	50	50
- ADENOCARCINOMA		-	1	-	-	-	-	-	1	-	-
SKIN	No.Examined	50	50	50	49	50	50	49	48	50	47
- HEMANGIOSARCOMA		-	-	-	-	1	-	-	-	-	-
- HEMANGIOMA		-	-	-	-	-	-	1	-	-	-
- SCHWANNOMA		-	1	-	-	-	-	1	1	1	1
- SQUAMOUS CELL CARCINOMA		-	-	-	-	-	-	1	-	-	-
- SARCOMA NOT OTHERWISE SPECIFIED		-	-	-	2	-	-	-	-	-	-

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STATUS AT NECROPSY: KO, INCL. DEATHS

ORGAN/FINDING	DOSE GROUP	A		B		C		D		E	
	SEX	M	F	M	F	M	F	M	F	M	F
	NO. ANIMALS	50	50	50	50	50	50	50	50	50	50
PANCREAS	No.Examined	50	50	49	49	50	50	49	50	48	46
- METASTATIC SARCOMA		-	-	-	-	-	-	-	-	1	-
KIDNEYS	No.Examined	50	50	49	49	50	50	49	50	49	49
- CORTICAL ADENOMA		-	1	-	-	1	-	-	-	-	-
- METASTATIC CARCINOMA		-	-	1	-	-	-	-	-	-	-
URINARY BLADDER	No.Examined	50	50	50	49	49	48	50	49	50	49
- LEIOMYOSARCOMA		-	-	1	-	-	-	-	-	-	-
TESTES	No.Examined	50	-	49	-	49	-	50	-	49	-
- LEYDIG CELL TUMOR		1	-	-	-	1	-	-	-	-	-
- HEMANGIOSARCOMA		1	-	-	-	-	-	-	-	-	-
SEMINAL VESICLES	No.Examined	50	-	50	-	50	-	49	-	49	-
- LEIOMYOSARCOMA		-	-	-	-	-	-	-	-	1	-
- LEIOMYOMA		1	-	-	-	-	-	-	-	-	-
- GRANULAR CELL TUMOR		-	-	1	-	-	-	-	-	1	-
OVARIES	No.Examined	-	50	-	48	-	49	-	48	-	48
- THECA/GRANULOSA CELL TUMOR		-	7	-	5	-	2	-	4	-	6
- LUTEOMA		-	1	-	2	-	2	-	1	-	3
- SERTOLI CELL TUMOR		-	-	-	1	-	-	-	-	-	-
- FIBROSARCOMA		-	-	-	-	-	-	-	1	-	-
UTERUS	No.Examined	-	50	-	46	-	50	-	49	-	49
- STROMAL SARCOMA		-	-	-	-	-	1	-	-	-	-
- LEIOMYOMA		-	1	-	2	-	-	-	-	-	-
- POLYP		-	4	-	2	-	1	-	-	-	-
- MYOMA		-	-	-	-	-	-	-	1	-	-
CERVIX	No.Examined	-	-	-	1	-	-	-	-	-	-
- LEIOMYOSARCOMA		-	-	-	1	-	-	-	-	-	-
PARATHYROID GLANDS	No.Examined	46	44	48	43	47	44	43	44	43	41
- ADENOMA		-	1	-	2	-	-	-	1	-	1

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NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

ORGAN/FINDING	DOSE GROUP	A		B		C		D		E	
	SEX	M	F	M	F	M	F	M	F	M	F
	NO. ANIMALS	50	50	50	50	50	50	50	50	50	50
BONE	No.Examined	50	50	50	50	50	50	49	50	50	50
- OSTEOSARCOMA		-	-	-	1	-	-	-	-	-	-
- OSTEOMA		-	1	-	-	-	-	-	-	-	-
BODY CAVITIES	No.Examined	1	-	2	2	4	-	3	1	5	-
- SARCOMA		-	-	-	-	-	-	-	-	1	-

C.6.b. Carcinogenicity in Rats

GLP Research Report #: Sponsor Volumes: 59-69

Conducted by :

Summary:

Tolcapone was administered in the diet at doses of 50, 250, and 450 mg/kg/day to Wistar rats (50/sex/dose group, 100/sex/control) for two years. Toxicokinetic analyses were conducted in satellite groups of 10 rats/sex/dose at weeks 4, 26, 52, 78 and 104.

Body weights were reduced by 22% in HDM, and 27% in HDF at study termination. Reductions in the MD groups did not exceed 7%. Food intake was significantly decreased in HDM from week 4/5 and HDF from week 2/3. Mortality occurred in all treatment groups at various times during the study; there were no clear drug-related effects.

The most notable non-neoplastic and neoplastic lesions were in kidney. Almost all (≥ 94%) HD rats and a large fraction of MD rats exhibited renal tubulopathy, tubular hyperplasia, and karyocytomegaly. Tubular hyperplasia with atypia was seen in 4-10% of MD and HD rats. Tubular cell carcinomas were diagnosed in 1 MDM, 3 HDM, and 1 HDF, and tubular cell adenomas were found in 2 MDF and 1 HDF. According to the sponsor and their expert outside evaluator, these tumors arise due to compensatory hyperplasia in response to tubule cell degeneration, and not because of a direct oncogenic effect of tolcapone. The degeneration is speculated to result from"metabolic overload" and exhaustion. The primary bases (among others) for this conclusion are:

1. tolcapone (and metabolites) are primarily excreted by the kidney
2. damage is restricted to the P3 segment which contains enzymes for xenobiotic metabolism
3. lipofuscin accumulation in the tubules is a sign of metabolic overload

The expert discounts karyomegaly as an indicator of a direct acting renal carcinogen, and cites several articles (including his own) to support this contention. In addition, he notes that the tumor formation was a late-occurring phenomenon requiring more than six-months of treatment, and that the compound was non-genotoxic. Renal tumors were found in the one-year study rat study (one nephroblastoma, one adenocarcinoma), and in the 13-week combination study with Sinemet (one nephroblastoma). The reviewer's interpretation of the genotoxicity data is that the compound was positive in the ML/TK assay.

Additional significant non-neoplastic findings were in the forestomach (squamous cell hyperplasia and hyperkeratosis) of MDM, HDM, and HDF. One HDM was diagnosed with a squamous cell carcinoma, and 2 HDM had squamous cell papillomas. The incidences of these tumors were considered within historical control range, but the high incidence of non-neoplastic lesions in this tissue in both rats and mice is consistent with a potentially neoplastic drug effect in this tissue. The sponsor suggests a direct local irritant effect of the drug as a causative factor. Forestomach changes are of questionable relevance to humans, which lack a forestomach, but since primate esophagus sometimes responds similarly to rodent forestomach (Greaves), these findings cannot be completely disregarded.

Endometrial hyperplasia was noted to occur at a higher incidence in tolcapone-treated rats. A dose relationship was not evident (highest occurrence in MD rats), so the finding was not indicated as significant by the sponsor. However, 8/60 HD rats were diagnosed with uterine adenocarcinomas, which was higher than the incidences in control (2/120), LD (3/60), and MD (3/60) rats. The incidence rate at the high dose (13.3%) exceeds all but one of the rates indicated in several historical control ranges. Coupled with the findings of endometrial hyperplasia, the possibility that these tumors are drug-related cannot be discounted. The fact that body weight in HDF was markedly suppressed, which may reduce the appearance of tumors, amplifies this possibility.

Toxicokinetic analyses suggested that plasma concentrations increased dose-proportionally, but levels did not stabilize until week 26 or 52 of treatment. Exposures in females rats appeared greater than in males. Relative to plasma exposures in humans receiving the projected maintenance dose of 200 mg, t.i.d., (AUC_{0-24} = 80 µg.hr/ml), tolcapone exposures in male and female rats were:

	Males	Females	
LD:	0.7 - 1.0	0.7 - 2.0	times the human exposure
MD:	3.4 - 7.2	4.1 - 14.5	"
HD:	6.3 - 14.2	8.6 - 32.0	"

The 50 mg/kg dose is considered the "No Effect" level.

Methods:

Dosages: 50, 250, 450 mg/kg/day (Batches 209 003 and 405 009).

Dose selection was based on both toxicological and pharmacokinetic considerations. Daily gavage administration of 300 mg/kg/day causes excessive mortality. The gavage route results in exposures that are approximately twice as high as exposure by feed admix. A dose of 500 mg/kg/day by feed-admix in a 6-month study was well tolerated, but caused a 10-20% reduction in body weight. A dose of 300 mg/kg/day did not affect body weight development. Thus, a dose of 450 mg/kg was selected to lie within this range and produce exposures that were 10-25 times the AUC following expected human doses (200 mg, t.i.d.).

Route of Administration: Drug-in-diet (pelleted preparation)
Species/Strain/Number: Wistar rat, Hannover-derived, SPF; 4 weeks old; males: 76-103g, females: 54-85 g
300/sex

mg/kg/day		Group 1 0	Group 2 0	Group 3 50	Group 4 250	Group 5 450
MALES	A	1- 50	61-110	121-170	181-230	241-290
	B	51- 60	111-120	171-180	231-240	291-300
FEMALES	A	301-350	361-410	421-470	481-530	541-590
	B	351-360	411-420	471-480	531-540	591-600

A - Oncogenicity Animals

B - Satellite Animals for Plasma Level Determinations at 4, 26, 52, 78 and 104 weeks.

Statistics: Routine analyses of body weight, food intake, organ weight and clinical data were by one-way ANOVA with a post-hoc Dunnett's test (normally distributed data) or Steel test (data not normally distributed). Fisher's exact test was used for macroscopic findings.

Statistical evaluation of neoplastic lesions was according to Peto et al. (1980) using the positive and negative trend tests with respect to dose. Only p-values <0.05 for rare neoplasms, and <0.01 for common neoplasms were considered statistically significant. The incidence of neoplastic lesions was considered significant only if the lesions occurred at a rate of 5% in any dose group and sex.

Results:

Mortality: No treatment-related effects on mortality were evident.

	Number of unscheduled deaths		Survival at week 104 Kaplan Meier Estimate	
	Males	Females	Males	Females
Group 1 (0 mg/kg/day)	15	14	77%	77%
Group 2 (0 mg/kg/day)	12	15	80%	75%
Group 3 (50 mg/kg/day)	11	18	83%	70%
Group 4 (250 mg/kg/day)	13(A)	12	81%	80%
Group 5 (450 mg/kg/day)	8	12	88%	80%

(A) = Two males died after blood sampling

Body Weight:

MALES

	WK 0	WK 105	% CON
C1	88	611	-
C2	87	611	-
50 mg/kg	87	602	98
250 "	87	574	94
450 "	87	474	78

FEMALES

	WK 0	WK 105	% CON
C1	68	339	-
C2	70	347	-
50 mg/kg	69	338	99
250 "	69	318	93
450 "	67	250	73

The first statistically significant reduction in body weight in HDM occurred at week 8 (5%). By week 20, HDM weighed 15% less than controls. HDF weighed 4% less by week 4, and 14% less by week 16. The weight differences between control an HD animals became progressively greater over the remainder of the study.

Some statistically significant differences in body weight between control and MD animals were evident by week 50, but since the differences never exceeded 11%, they are not considered important.

Food Intake:

Significantly decreased at most points during the study in HDM (4-14%) and HDF (7-20%).

Hematology: (differential leukocyte count on control and HD at week 103)

No treatment-related effects were evident. Individual variations outside of a historical reference range were:

↑ lymphocytes	-	1 Con F
↓ lymphocytes	-	1 HDM, 1 HDF
↑ Segs	-	2 HDM
↑ eosinophils	-	2 Con M, 1 Con F
		2 HDM, 1 HDF
↑ monocytes	-	4 Con M, 3 Con F
		6 HDM, 3 HDF

Gross Pathology:

lung, foci	-	MDM (35%)
stomach,		
discoloration	-	MDM (13%), HDM (30%)
		HDF (23%)
foci	-	HDM (42%)
		HDF (20%)
duodenum, dilatation	-	HDF (10%)
cecum,		
liquid contents	-	HDM (15%)
distended w/ feces	-	HDM (22%)
		HDF (10%)
kidney, watery cyst	-	HDF (12%)
uterus, discoloration	-	MDF (30%)
thymus, foci	-	HDF (13%)
mesenteric lymph node,		
nodular thickening	-	MDM (8%), HDM (8%)

Non-neoplastic lesions:

Lesion			Group/Incidence Rate (%) 1	2	3	4	5
forestomach	squamous cell hyperplasia	M	2	-	4	39*	88*
		F	2	-	4	4	28*
	hyperkeratosis	M	2	-	4	28*	70*
		F	-	-	2	4	14*
kidney	cortical cysts	M	8	4	6	4	6
		F	2	2	4	8	36*
	karyocytomegaly	M	-	-	-	43*	92*
		F	-	-	-	90*	95*
	papillary hyaline casts	M	4	16	10	26*	56*
		F	4	2	4	20*	38*
	papillary degeneration	M	-	-	-	6*	21*
		F	-	-	-	18*	16*
	tubular hyperplasia	M	-	-	-	33*	90*
		F	-	-	-	78*	89*
	tubular hyperplasia w/ atypia	M	-	-	-	4+	8+
		F	-	-	-	6+	9+
	tubular cystic hyperplasia	M	-	-	-	2	15
		F	-	-	-	27	73
	tubular necrosis	M	-	-	-	18*	38*
		F	-	-	2	47*	85*
	tubular hypertrophy	M	-	-	-	-	2
		F	2	-	-	2	11
	tubular cyto. lipofuscin	M	-	-	-	-	-
		F	-	-	-	31	60
	tubulopathy	M	-	-	-	78*+	98*+
		F	-	-	-	96*+	95*+
uterus	cystic endometrial hyperplasia		8	12	15	30	14

* The statistically significant effects were not clearly identified in the summary tables or in a statistical table. Thus, only those effects described in the text as "significant" are labeled.

\+ Shown are data from the Expert Re-evaluation (Vol. 72:69).

The data include animals from both the oncogenicity and toxicokinetic groups. The TK animals were examined microscopically only when there was a necropsy finding. This resulted in unequal *n's* among groups; hence, the data are expressed as "Incidence Rates".

Tubulopathy: a summarizing term used by the pathologist when either tubular cell degeneration, tubular single cell necrosis, tubular cell hyperplasia, and/or karyocytomegaly was diagnosed in the straight portion of the proximal tubules.

According to the sponsor's expert evaluation of the data, the basic renal lesion was single cell death in the straight portion of the proximal tubule at the mid and high dose level. The tubulopathy was characterized by tubular cell degeneration, necrosis with reactive regeneration and hyperplasia occasionally with atypia, and karyocytomegaly. Females tended to be more severely affected than males, possibly due to higher drug exposures. The suggestion is raised that cell death and "dropping out" (degenerate cells were found in the tubule lumen) may be due to "metabolic overload" or exhaustion; the accumulation of cytoplasmic lipofuscin, an index of increased metabolic activity, is consistent with this hypothesis. The compensatory hyperplasia is suggested as a mechanism of renal tumor development. The expert suggests that karyomegaly is not necessarily indicative of a direct renal oncogenic effect of tolcapone.

The forestomach changes were marked and appear dug-related, particularly in males. The sponsor suggest a direct local irritant effect of the drug as a causative factor. These findings support the contention that the observed stomach neoplasias are also drug-related. Forestomach changes are of questionable relevance to humans, which lack a forestomach, but since primate esophagus sometimes responds similarly to rodent forestomach (Greaves), these findings cannot be completely disregarded.

Increased incidences of endometrial hyperplasia were not dose-related, and were thus not considered significant. However, this lesion appeared more frequently in drug-treated animals, and is mentioned in view of its possible relationship with uterine tumors. The sponsor suggests that estrogenic effects of tolcapone may underlie this lesion, but no data was submitted to support this mechanism.

Neoplastic lesions

Lesion			1	2	3	4	5
kidney	tubular cell carcinoma	M	0/50	0/51	0/52	1/51	3/52
		F	0/50	0/50	0/50	0/50	1/55+
	tubular cell adenoma	M	0/50	0/51	0/52	0/51	0/52
		F	0/50	0/50	0/50	2/50	1/55+
stomach	squamous cell carcinoma	M	0/52	0/50	0/51	0/53	1/55
		F	0/50	0/52	0/50	0/51	0/55
	squamous cell papilloma	M	0/52	0/50	0/51	0/53	2/55
		F	0/50	0/52	0/50	0/51	0/55
	adenoma	M	0/52	0/50	0/51	0/53	1/55
		F	0/50	0/52	0/50	0/51	0/55
uterus	adenocarcinoma		0/53	2/57	3/54	3/53	8/57

\+ Shown are data from the Expert Re-evaluation (Vol. 72:69).

The renal tumors were considered to be due to increased tubular cell proliferation as a consequence of tubular necrosis.

The apparent dose-related increase in uterine tumors suggests that this may be a true effect of the drug. The sponsor has submitted two documents with historical control data for uterine adenocarcinomas in European Wistar rats. In a book chapter by Brown and Leininger (Pathobiology of the Aging Rat, 1992), a very broad range of incidence values is reported (0.5, 1.3, 5, 11, 12.7, 39%). In an EPS (Experimental Pathology Services) historical control data compilation, the highest incidence of uterine adenocarcinomas in 27 studies of 104-130 weeks duration was 8%. Since the incidence rate in this study (8/57 = 14%) exceeds all but one of the historical control data points, it should not be dismissed on this basis. The fact that body weight in HDF was markedly suppressed, and hyperplastic changes in the uterus were also noted at a relatively high frequency adds further support for the contention that TASMAR may be responsible for uterine neoplastic changes in the rat.

The incidence of forestomach tumors was also considered within historical control range. However, the high incidence of non-neoplastic lesions in this tissue in both rats and mice is consistent with a potentially neoplastic drug effect in this tissue.

Decreased incidences of pituitary adenomas in HDM and HDF, and mammary gland fibroadenomas in HDF were also noted.

Plasma Concentrations: (weeks 4, 26, 52, 78, 104 at 0700, 1100, 1600, 2100, 0200 (from 2/sex/dose/time point)

Increases in plasma concentrations were approximately dose-proportional at the initial measurement (week 4). From weeks 4-52, increases were greater than dose proportional (two-fold in males, three-fold in females), and stabilized thereafter. Plasma levels were two- to three-fold higher in females compared to males.

Based on AUC determinations made between weeks 52-104, rat exposures were higher than exposures in humans receiving 200 mg, t.i.d. (80 ng.hr/ml), by 12-14 times in HDM, and 24-32 times in HDF.

Table 1 : Pharmacokinetic parameters of Ro 40-7592 in male rats

	Cmax (µg/ml)				
	week 4	week 26	week 52	week 78	week 104
Group 3	3.07	3.60	4.07	4.17	4.24
Group 4	16.1	27.9	29.4	29.4	27.2
Group 5	28.0	53.1	58.9	50.0	53.7

Table 2 : Pharmacokinetic parameters of Ro 40-7592 in female rats

	Cmax (µg/ml)				
	week 4	week 26	week 52	week 78	week 104
Group 3	3.21	7.04	6.66	7.08	9.42
Group 4	27.1	37.3	61.2	55.7	59.2
Group 5	51.3	82.2	126	102	182

Dosage group 3 : 50 mg/kg Ro 40-7592
Dosage group 4 : 250 mg/kg Ro 40-7592
Dosage group 5 : 450 mg/kg Ro 40-7592

Table 3 : Pharmacokinetic parameters of Ro 40-7592 in male rats

	AUC 0-24 (h.µg/ml)				
	week 4	week 26	week 52	week 78	week 104
Group 3	54.5	72.4	76.5	71.7	72.7
Group 4	274	458	573	508	475
Group 5	503	989	1138	956	1041

Table 4 : Pharmacokinetic parameters of Ro 40-7592 in female rats

	AUC 0-24 (h.µg/ml)				
	week 4	week 26	week 52	week 78	week 104
Group 3	56.3	118	122	128	166
Group 4	328	763	1161	978	889
Group 5	691	1487	2507	1882	2563

Dosage group 3 : 50 mg/kg Ro 40-7592
Dosage group 4 : 250 mg/kg Ro 40-7592
Dosage group 5 : 450 mg/kg Ro 40-7592

TEST ARTICLE : RO 40-7592/001
TEST SYSTEM : RAT, 104 WEEKS, FEED ADMIXTURE
SPONSOR : F.HOFFMANN-LA ROCHE AG
PATHOL. NO.: 95005 HUN
DATE : 17-APR-96
PathData9 System V4.1

% OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
: AT NECROPSY: RO, INCL. DEATHS
Carcinogenicity Groups A

ORGAN/FINDING	DOSE GROUP / SEX / NO. ANIMALS	1A M 50	1A F 50	2A M 50	2A F 50	3A M 50	3A F 50	4A M 50	4A F 50	5A M 50	5A F 50
BRAIN	No.Examined	50	50	50	50	50	50	50	50	50	49
- Astrocytoma		1	-	-	-	1	-	-	-	-	-
- Granular cell tumor		1	1	-	-	-	-	1	-	2	-
- Malignant reticulosis		-	-	-	-	-	1	-	1	-	-
- Metastatic carcinoma		-	-	-	-	-	1	-	-	-	-
- Oligodendroglioma		-	-	-	-	-	-	-	-	1	-
HEART	No.Examined	50	49	50	50	50	49	50	49	50	49
- Schwannoma		-	-	-	-	1	-	-	-	-	-
LUNG	No.Examined	49	49	50	50	50	49	50	49	50	49
- Alveolar/bronchiolar adenoma		1	-	-	-	-	-	-	-	-	-
- Metastatic carcinoma		-	-	-	-	-	1	-	-	-	-
- Metastatic sarcoma		1	-	-	-	-	-	-	-	-	-
ORAL CAVITY	No.Examined	-	-	-	-	-	1	-	-	1	-
- Squamous cell papilloma		-	-	-	-	-	1	-	-	-	-
- Squamous cell carcinoma		-	-	-	-	-	-	-	-	1	-
STOMACH	No.Examined	50	50	50	50	50	50	50	49	50	50
- Squamous cell carcinoma		-	-	-	-	-	-	-	-	1	-
- Squamous cell papilloma		-	-	-	-	-	-	-	-	2	-
DUODENUM	No.Examined	50	49	50	50	50	50	50	49	49	50
- Adenocarcinoma		-	-	1	-	-	-	-	-	-	-
- Leiomyoma		-	-	-	1	-	-	-	-	-	-
COLON	No.Examined	50	49	50	50	50	50	50	49	49	50
- Sarcoma (not otherwise specified)		-	-	-	-	-	1	-	-	-	-
LIVER	No.Examined	50	50	50	50	50	50	49	49	50	50
- Hemangioma		-	-	-	-	-	1	-	1	1	-
- Hemangiosarcoma		1	-	-	-	-	-	-	-	-	-
- Hepatocellular adenoma		3	-	3	1	2	1	1	-	3	-

TEST ARTICLE : RO 40-7592/001
TEST SYSTEM : RAT, 104 WEEKS, FEED ADMIXTURE
SPONSOR : F.HOFFMANN-LA ROCHE AG
PATHOL. NO.: 95005 HUN
DATE : 17-APR-96
PathData9 System V4.1

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: RO, INCL. DEATHS
Carcinogenicity Groups A

ORGAN/FINDING	DOSE GROUP / SEX / NO. ANIMALS	1A M 50	1A F 50	2A M 50	2A F 50	3A M 50	3A F 50	4A M 50	4A F 50	5A M 50	5A F 50
PANCREAS	No.Examined	49	50	50	50	50	46	50	49	50	50
- Acinar cell adenoma		3	-	1	-	-	-	-	-	-	-
- Islet cell adenoma		1	-	3	-	5	1	4	-	-	-
- Islet cell carcinoma		-	-	1	-	-	-	-	-	-	-
KIDNEYS	No.Examined	50	50	50	50	50	50	50	49	50	50
- Lipomatous tumor		1	-	2	1	-	-	-	-	1	-
- Tubular cell carcinoma		-	-	-	-	-	-	-	1	-	2
- Tubular cell adenoma		-	-	-	-	-	-	-	2	-	2
TESTES	No.Examined	50	-	50	-	50	-	50	-	50	-
- Benign Leydig cell tumor		2	-	1	-	1	-	3	-	-	-
OVARIES	No.Examined	-	50	-	50	-	49	-	49	-	50
- Benign granulosa-theca cell tumor		-	1	-	-	-	1	-	-	-	1
- Benign granulosa cell tumor		-	2	-	1	-	1	-	-	-	2
- Benign Sertoli cell tumor		-	-	-	-	-	-	-	-	-	1
- Benign thecoma		-	-	-	-	-	1	-	-	-	-
UTERUS	No.Examined	-	50	-	50	-	49	-	50	-	50
- Adenocarcinoma		-	-	-	-	-	2	-	3	-	7
- Adenoma		-	1	-	-	-	-	-	-	-	2
- Carcinosarcoma		-	-	-	1	-	-	-	-	-	-
- Hemangioma		-	-	-	-	-	-	-	1	-	-
- Leiomyoma		-	1	-	1	-	-	-	-	-	-
- Leiomyosarcoma		-	-	-	-	-	-	-	-	-	1
- Stromal polyp		-	7	-	6	-	4	-	5	-	3
- Benign teratoma		-	-	-	-	-	-	-	-	-	1
CERVIX	No.Examined	-	50	-	50	-	49	-	50	-	50
- Schwannoma		-	-	-	-	-	2	-	-	-	-
- Stromal polyp		-	1	-	-	-	-	-	-	-	-
PITUITARY GLAND	No.Examined	50	50	50	50	50	50	49	50	50	49
- Adenoma of pars distalis		12	25	10	26	13	23	13	23	3	19
- Adenoma of pars intermedia		-	-	1	-	-	-	1	1	-	-

TEST ARTICLE : RO 40-7592/001
TEST SYSTEM : RAT, 104 WEEKS, FEED ADMIXTURE
SPONSOR : F.HOFFMANN-LA ROCHE AG
PATHOL. NO.: 95005 HUN
DATE : 17-APR-96
PathData9 System V4.1

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: RO, INCL. DEATHS
Carcinogenicity Groups A

ORGAN/FINDING	DOSE GROUP / SEX / NO. ANIMALS	1A M 50	1A F 50	2A M 50	2A F 50	3A M 50	3A F 50	4A M 50	4A F 50	5A M 50	5A F 50
MAMMARY GLAND	No.Examined	50	50	50	50	50	50	50	50	50	49
- Adenocarcinoma		-	3	1	3	-	3	-	-	-	1
- Adenoma		-	-	-	-	-	-	2	-	-	-
- Fibroadenoma		8	8	-	12	-	7	-	9	-	4
SKIN/SUBCUTIS	No.Examined	50	50	50	50	50	50	50	50	50	49
- Basal Cell Tumor		1	-	1	-	1	-	-	-	1	-
- Fibroma		1	1	1	-	1	-	2	-	2	-
- Keratoma		1	-	-	-	-	-	-	-	-	-
- Hemangioma		1	-	2	-	-	-	-	-	1	-
- Keratoacanthoma		2	-	3	-	4	-	-	-	-	-
- Leiomyosarcoma		-	1	-	-	-	-	-	-	-	-
- Lipoma		1	-	-	-	1	-	-	-	-	-
- Metastatic sarcoma		-	-	-	-	-	-	-	-	1	-
- Schwannoma		-	1	1	-	-	-	-	-	-	-
- Schwannoma squamous cell carcinoma		-	-	-	-	1	-	-	-	-	-
- Squamous cell carcinoma		-	-	1	-	1	1	-	-	-	-
- Squamous cell papilloma		1	-	-	-	-	1	-	-	-	-
BONE (OTHER)	No.Examined	1	1	-	-	1	1	-	-	-	-
- Osteoma		1	-	-	-	-	-	-	-	-	-
	No.Examined	-	1	-	2	1	-	3	-	-	-
- Adrenal cortical tumor		-	-	-	1	-	-	1	-	-	-
BODY CAVITIES	No.Examined	4	3	4	5	3	7	3	4	5	2
- Fibrosarcoma		-	-	-	-	-	-	-	-	1	-
- Lipoma		-	-	-	1	-	-	-	-	-	-
- Mesothelioma		1	1	-	-	-	1	-	-	-	-
- Schwannoma		-	-	-	1	-	-	-	-	-	-

TEST ARTICLE : RO 40-7592/001
TEST SYSTEM : RAT, 104 WEEKS, FEED ADMIXTURE
SPONSOR : F.HOFFMANN-LA ROCHE AG
PATHOL. NO.: 95005 HUN
DATE : 17-APR-96
PathData9 System V4.1

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: RO, INCL. DEATHS
Carcinogenicity Groups A

ORGAN/FINDING	DOSE GROUP / SEX / NO. ANIMALS	1A M 50	1A F 50	2A M 50	2A F 50	3A M 50	3A F 50	4A M 50	4A F 50	5A M 50	5A F 50
THYROID GLAND	No.Examined	50	49	50	50	50	48	50	49	48	48
- C-cell adenoma		4	7	4	4	3	5	4	5	9	
- Follicular cell adenoma		-	-	1	-	-	-	1	-	-	-
PARATHYROID GLANDS	No.Examined	49	47	50	46	50	48	47	47	47	43
- Adenoma		3	1	2	-	1	-	3	1	-	1
ADRENAL CORTICES	No.Examined	50	50	50	50	50	50	50	49	50	50
- Adenocarcinoma		-	-	-	-	-	-	-	-	-	1
- Adenoma		-	2	1	-	-	-	-	-	-	-
ADRENAL MEDULLAE	No.Examined	50	50	50	50	50	50	50	49	50	49
- Benign pheochromocytoma		3	-	3	-	1	-	1	1	2	1
- Malignant pheochromocytoma		1	-	-	-	-	-	-	-	1	-
ISLET, PANCREAT. ADE.	No.Examined	50	50	50	50	50	50	50	50	50	50
- Lymphoblastic malignant lymphoma		1	-	-	1	-	-	-	-	-	-
- Lymphocytic malignant lymphoma		1	1	1	2	3	2	1	2	-	2
- Malignant fibrous histiocytoma		1	-	-	-	-	-	-	-	-	-
SPLEEN	No.Examined	50	50	50	50	50	50	50	49	50	50
- Hemangiosarcoma		1	-	-	-	1	-	-	-	-	-
THYMUS	No.Examined	47	49	47	50	49	48	49	49	49	49
- Benign thymoma		-	2	-	-	-	-	-	-	-	-
LYMPH NODES	No.Examined	3	5	4	4	5	4	2	5	6	8
- Hemangioma		-	-	-	-	1	-	-	-	-	-
- Metastatic carcinoma		-	-	-	-	-	-	-	1	-	-
- Metastatic sarcoma		-	2	-	-	-	-	-	-	1	-
MESENT. LYMPH NODE	No.Examined	50	49	50	50	50	50	50	49	50	49
- Hemangioma		4	4	4	2	7	-	7	-	1	2

TEST ARTICLE : BO 40-7892/001
TEST SYSTEM : RAT, 104 WEEKS, FEED ADMIXTURE
SPONSOR : F.HOFFMANN-LA ROCHE AG

PATHOL. NO.: 95006 RHH
DATE : 17-APR-96
PathData9 System V4.1

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS
Satellite Groups B

ORGAN/FINDING	DOSE GROUP	10		20		30		40		50	
	SEX	M	F	M	F	M	F	M	F	M	F
	NO. ANIMALS	10	10	10	10	10	10	10	10	10	10
BRAIN	No.Examined	2	5	1	4	1	4	1	4	-	3
- Granular cell tumor		-	-	-	-	1	-	1	-	-	-
- Oligodendroglioma		-	-	-	-	-	1	-	-	-	-
LUNG	No.Examined	6	2	3	6	3	3	3	4	1	3
- Alveolar/bronchiolar adenoma		-	-	-	-	-	-	1	-	-	-
- Metastatic uf carcinoma		1	-	-	-	-	-	-	-	-	-
STOMACH	No.Examined	2	-	-	2	1	-	3	1	5	3
- Adenoma		-	-	-	-	-	-	-	-	1	-
DUODENUM	No.Examined	-	-	-	1	-	-	-	-	-	2
- Leiomyoma		-	-	-	1	-	-	-	-	-	-
LIVER	No.Examined	1	4	3	1	2	-	4	1	3	-
- Hepatocellular carcinoma		-	-	-	-	-	-	-	-	1	-
PANCREAS	No.Examined	-	1	1	-	2	-	-	-	1	-
- Acinar cell adenoma		-	-	-	-	1	-	-	-	-	-
KIDNEYS	No.Examined	-	-	1	-	2	-	1	1	2	5
- Tubular cell carcinoma		-	-	-	-	-	-	-	-	1	-
TESTES	No.Examined	1	-	2	-	2	-	2	-	2	-
- Benign Leydig cell tumor		-	-	-	-	1	-	1	-	1	-
OVARIES	No.Examined	-	1	-	2	-	4	-	2	-	4
- Benign granulosa-theca cell tumor		-	-	-	-	-	1	-	-	-	-
UTERUS	No.Examined	-	3	-	7	-	5	-	3	-	7
- Adenocarcinoma		-	-	-	2	-	1	-	-	-	1
- Stromal polyp		-	-	-	-	-	1	-	2	-	1
PITUITARY GLAND	No.Examined	2	7	2	7	2	8	2	5	-	3
- Adenoma of pars distalis		2	7	1	7	-	8	-	5	-	3
- Adenoma of pars intermedia		-	-	1	-	-	-	1	-	-	-

TEST ARTICLE : BO 40-7892/001
TEST SYSTEM : RAT, 104 WEEKS, FEED ADMIXTURE
SPONSOR : F.HOFFMANN-LA ROCHE AG

PATHOL. NO.: 95006 RHH
DATE : 17-APR-96
PathData9 System V4.1

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS
Satellite Groups B

ORGAN/FINDING	DOSE GROUP	10		20		30		40		50	
	SEX	M	F	M	F	M	F	M	F	M	F
	NO. ANIMALS	10	10	10	10	10	10	10	10	10	10
THYROID GLAND	No.Examined	-	-	-	-	-	-	1	-	1	-
- Follicular cell adenoma		-	-	-	-	-	-	1	-	1	-
ADRENAL MEDULLA	No.Examined	4	8	3	-	4	2	1	1	2	2
- Benign pheochromocytoma		-	-	1	-	-	-	-	-	-	-
- Malignant pheochromocytoma		1	-	1	-	-	-	-	-	-	1
HEMOLYMPHORET. SYS.	No.Examined	-	-	1	1	-	-	1	-	-	-
- Lymphoblastic malignant lymphoma		-	-	-	-	-	-	1	-	-	-
- Lymphocytic malignant lymphoma		-	-	1	1	-	-	-	-	-	-
TONGUE	No.Examined	3	-	-	3	-	1	-	-	-	1
- Squamous cell carcinoma		1	-	-	-	-	-	-	-	-	-
HEART, LYMPH NODE	No.Examined	1	-	4	1	1	-	1	-	3	-
- Hemangioma		1	-	2	1	1	-	-	-	1	-
- Hemangiosarcoma		-	-	1	-	-	-	-	-	-	-
MAMMARY GLAND	No.Examined	1	6	-	6	-	8	-	5	-	1
- Adenocarcinoma		-	2	-	-	-	2	-	-	-	-
- Fibroadenoma		-	4	-	3	-	4	-	2	-	-
SKIN/SUBCUTIS	No.Examined	2	5	3	3	1	3	1	2	2	-
- Fibroma		1	1	1	-	-	-	-	-	-	-
- Keratoacanthoma		-	-	-	-	1	-	-	-	-	-
- Sebaceous		-	-	-	-	-	-	1	-	-	-
- Squamous cell papilloma		-	-	1	-	-	-	-	-	-	-

END OF REPORT SECTION

C.7. Antigenicity in Mice and Guinea Pigs

Conducted by : Department of Toxicology and Pathology
Nippon Roche Research Center
Kamakura, Japan

Research Report #: J-146,090

Summary:

Tolcapone did not display antigenic properties in the active systemic anaphylaxis in Guinea pigs and passive cutaneous anaphylaxis in rats.

Methods:

Active Systemic Anaphylaxis (ASA) in Guinea Pigs:

Two groups (n = 10) were used to assess the antigenicity of tolcapone and Penicillin G as a positive control. Animals were sensitized by two subcutaneous injections of either tolcapone (7.5 mg) linked to guinea pig serum albumin (GPSA), or penicillin (7.5 mg) linked to GPSA at 4 day intervals. Animals were challenged after 10 or 20 days (n=5/day) by intravenous of either tolcapone or penicillin linked to mouse serum albumin (MSA; 12 mg/animal). Animals were monitored for anaphylaxis.

Passive Cutaneous Anaphylaxis (PCA) in Rats:

Groups of 10, 10, and 5 mice were used to prepare tolcapone, penicillin or ovalbumin antigenic sensitization solutions. Each compound was linked to GPSA, diluted in saline and mixed with alum cream at a concentration to apply 10 or 1 μg/of tolcapone or penicillin per animal. Animals were sensitized by two intraperitoneal injections of either tolcapone-GPSA, penicilli-GPSA or ovalbumin at 4 week intervals. Two weeks after the final injection, blood was collected from mice by cardiac puncture for preparation of antisera, which was injected intradermally into rats. Twenty-four hrs later, the rats were challenged with an intravenous injection of tolcapone or penicillin linked to mouse serum albumin (MSA), or ovalbumin solution containing Evans' blue dye. Thirty minutes after the challenge, the rats were sacrificed and evaluated for a reaction by the formation of blue spots in the skin.

Results:

Tolcapone did not produce signs of anaphylaxis in sensitized guinea pigs. Signs of anaphylaxis were observed in the penicillin-sensitized positive control groups.

In the PCA test, no blue spots were observed in the skin of rats that were sensitized and challenged with tolcapone. Penicillin produced the expected antigenic response.

D. PHARMACOKINETIC/ADME STUDIES

Single Dose Pharmacokinetics/Absorption

1. Single-Dose Oral and IV Pharmacokinetics in Rats and Dogs
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D.1. Single-Dose Oral and IV Pharmacokinetics in Rats and Dogs
Research Report #: B-104,522 Volume: 79

Summary:

The single-dose pharmacokinetics of tolcapone, including the formation of 3-O-methyltolcapone, were determined in rats (i.v.: 1.5 mg/kg; p.o.: 1.5 and 29.7 mg/kg) and dogs (i.v.: 1.5 and 2.0 mg/kg; p.o.: 1.8 and 4.3 mg/kg). The pharmacokinetics of 3-O-methyltolcapone were also determined following administration to rats (0.5 mg/kg, i.v.) or dogs (0.5-1.0 mg/kg, i.v.; 1.7-2.0, p.o.).

The pharmacokinetics of tolcapone were similar in the two species. Tolcapone had a relatively short half-life, low volume of distribution, low plasma clearance, and high oral bioavailability.

Results:

Single-Dose Tolcapone Pharmacokinetics in **Rats**

Dose, route	t_{max} (hr)	C_{max} hr	$AUC_{(0\text{-}inf)}$ (h.µg/ml)	Vd_{ss} (l/kg)	Cl (ml/min.kg)	$t_{1/2}\beta$ (hr)	F
1.5, i.v.	.		2.91	0.20	8.61	0.56	
1.5, p.o.	0.33	1.41	2.19			1.66*	74.6
29.7, p.o.	0.47	10.45	26.73				

*median; n = 3/group

Single-Dose Tolcapone Pharmacokinetics in **Dogs**

Dose, route	t_{max} (hr)	C_{max} hr	$AUC_{(0\text{-}inf)}$ (h.µg/ml)	Vd_{ss} (l/kg)	Cl (ml/min.kg)	$t_{1/2}\beta$ (hr)	F
1.5, i.v.			16.13	0.16	1.54	1.76	
2.0, i.v.			14.86	0.16	2.24	1.13	
1.8, p.o.	1.5	5.04	14.00			1.39	71.4
4.3, p.o.	0.5	8.27	20.00			1.13	62.2

n = 1

The 3-O-methyl metabolite appeared rapidly in plasma after i.v. administration in both species, although t_{max} was achieved relatively slowly in dogs. Plasma levels of metabolite were generally low, although in rats they exceeded those of the parent by 1 hr post-dose. Elimination of the metabolite was slower than that of the parent compound:

3-O-Methyltolcapone Pharmacokinetics after Tolcapone Administration in **Rats**

TOL dose, route	t_{max} (hr)	C_{max} (hr)	$AUC_{(0\text{-}inf)}$ (h.μg/ml)	Vd_{ss} (l/kg)	Cl (ml/min.kg)	$t_{1/2}\beta$ (hr)
1.5, i.v.		0.64	1.64			0.24
1.5, p.o.	0.33	0.50	2.80			2.87
29.7, p.o.	1.0	0.59	10.75			11.9

3-O-Methyltolcapone Pharmacokinetics after Tolcapone Administration in **Dogs**

TOL dose, route	t_{max} (hr)	C_{max} (hr)	$AUC_{(0\text{-}inf)}$ (h.μg/ml)	Vd_{ss} (l/kg)	Cl (ml/min.kg)	$t_{1/2}\beta$ (hr)
1.5, i.v.	5.0	0.90	19.20			10.4
2.0, i.v.	4.0	0.42	6.48			7.83
1.8, p.o.	7.0	0.79	16.00			9.17
4.33, p.o.	5.0	0.56	7.92			3.95

n = 1

The **single-dose pharmacokinetic parameters for the 3-O-methyl-metabolite** were determined following intravenous and oral administration, and were not markedly different from those of the parent compound:

Rats

3-O-MeTOL dose, route	t_{max} (hr)	C_{max} (hr)	$AUC_{(0\text{-}inf)}$ (h.μg/ml)	Vd_{ss} (l/kg)	Cl (ml/min.kg)	$t_{1/2}\beta$ (hr)
0.5, i.v.			14.90	0.22	1.69	1.72

n = 3 rats/group

Dogs

3-O-MeTOL dose, route	t_{max} (hr)	C_{max} (hr)	$AUC_{(0\text{-}inf)}$ (h.μg/ml)	Vd_{ss} (l/kg)	Cl (ml/min.kg)	$t_{1/2}\beta$ (hr)	F
0.5, i.v.			26.76	0.19	0.32	7.46	
1.0, i.v.			30.15	0.24	0.55	6.26	
1.7, p.o.	0.67	7.00	29.06			2.59	57.3
2.0, p.o	1.50	8.40	54.6			7.80	86.8

D.2. Single-dose Pharmacokinetics of ^{14}C-Tolcapone in Rats

Research Report # J-146,430 Volume: 79

Summary:

The single-dose pharmacokinetics of ^{14}C-tolcapone were determined in male rats administered 5, 20, 100 mg/kg, p.o., or 5 mg/kg, i.v., and female rats administered 20 mg/kg, p.o. Groups consisted of 4 rats.

Following oral administration of ^{14}C-tolcapone to males, increases in Cmax of total radiolabel, parent compound, or metabolite were less than dose-proportional (Sponsor Table 1). Increases in AUC of the radiolabel and the parent compound were dose-proportional. Tmax increased with dose. The half-life of the parent compound was much shorter than the half-life of the total radiolabel. The bioavailability of tolcapone was moderate (55.0-58.5), and that of the radiolabel was slightly higher (64-70%), suggesting low first-pass metabolism. The Cmax of 3-O-methyltolcapone was 2.5-5% of the Cmax of the parent compound.

No significant gender differences were evident.

Table 1 Pharmacokinetic parameters of ^{14}C-radioactivity, Ro40-7592 and Ro40-7591 in blood after single oral and intravenous administration of ^{14}C-Ro40-7592 to rats

	Sex	Dose (mg/kg)	Route	Pharmacokinetic parameters Tmax (hr)	Cmax (μg eq./ml)	T1/2 (hr)	AUC(0-∞) (μg eq.*hr/ml)	B.A. (%)
^{14}C-radioactivity	Male	5	i.v.	-	-	11.79 ± 0.69	16.33 ± 0.91	
		5	p.o.	0.38 ± 0.07	6.88 ± 0.67	11.92 ± 1.21	10.77 ± 0.26	
		20	p.o.	0.63 ± 0.07	17.53 ± 3.20	22.20 ± 3.91	42.02 ± 3.16	
		100	p.o.	2.88 ± 0.83	36.88 ± 3.13	15.90 ± 2.34	230.25 ± 18.76	
	Female	20	p.o.	0.63 ± 0.13	19.53 ± 1.10	32.98 ± 4.77	49.85 ± 6.58	
Ro40-7592	Male	5	i.v.	-	-	0.65 ± 0.02	9.51 ± 0.40	(100)
		5	p.o.	0.31 ± 0.06	4.92 ± 0.76	0.93 ± 0.04	5.34 ± 0.23	56.2
		20 *	p.o.	0.50 ± 0.14	12.31 ± 2.62	1.15 ± 0.15	22.27 ± 2.59	58.5
		100	p.o.	2.63 ± 0.85	23.88 ± 2.33	1.59 ± 0.41	104.53 ± 14.21	55.0
	Female	20 *	p.o.	0.50 ± 0.00	13.75 ± 0.58	0.82 ± 0.02	26.14 ± 2.93	
Ro40-7591	Male	5	i.v.	-	-	N.C.	N.C.	
		5	p.o.	0.56 ± 0.16	0.24 ± 0.01	N.C.	N.C.	
		20 *	p.o.	0.67 ± 0.08	0.31 ± 0.03	N.C.	N.C.	
		100	p.o.	2.13 ± 0.72	0.85 ± 0.15	N.C.	N.C.	
	Female	20 *	p.o.	0.42 ± 0.08	0.30 ± 0.03	N.C.	N.C.	

Data are expressed as the mean values±S.E. of four animals. (* : three animals)
N.C. : Not calculated

D.3. Tissue distribution following single oral administration of ^{14}C-Tolcapone (5 mg/kg) to pigmented rats

Research Report #: B-113,223 Volume: 80

Summary:

The tissue distribution of 5 mg/kg ^{14}C-tolcapone was determined in pigmented (piebald) rats (12 M, 12 F) at 0.5-48 hrs after oral administration. Concentrations of radioactivity were determined by scintillation counting.

Maximum levels of radioactivity in tissues were attained at 0.5 hr post-dose (Sponsor Tables 2&3). Highest concentrations were achieved in the in the organs of absorption (gut) and elimination (liver, kidney). The slowest rate of decline in radioactivity levels were in the liver, kidney, and gut. The highest amount of radioactivity detected in brain was 0.04 times the blood level. By 48 hrs, significant levels (0.5 µg equiv/g tissue) were detectable only in the stomach, intestine, kidney, liver, and white skin {the 48 hr measurement in male brain tissue is considered spurious by the reviewer}. A tendency for higher levels in females was noted. This finding could not be statistically evaluated due to the low sample size, but would not be unexpected based on toxicokinetic studies demonstrating higher tolcapone plasma levels in females.

Table 2

Tissue distribution of Ro 40-7592 (µg-equiv./g tissue) in pigmented male piebald rats following a single dose of 5 mg/kg of ^{14}C-labelled Ro 40-7592

Tissue / organ	Time (hours) 0.5[a]	2[a]	4[a]	7[a]	24[a]	48[a]
blood[b]	3.22 ± 0.40	2.01 ± 0.20	0.81 ± 0.06	0.20 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
heart	0.75 ± 0.02	0.00 ± 0.01	0.00 ± 0.04	0.10 ± 0.05	0.01 ± 0.00	0.00 ± 0.00
lungs	1.20 ± 0.30	0.00 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.00	0.00 ± 0.00
stomach	60.41 ± 05.00	0.70 ± 0.01	0.00 ± 0.00	0.00 ± 1.00	0.05 ± 0.11	0.00 ± 0.02
intestine	2.72 ± 0.01	0.10 ± 0.00	0.01 ± 0.00	00.07 ± 1.00	4.00 ± 1.00	1.07 ± 0.12
kidney	0.07 ± 1.00	4.04 ± 0.27	0.11 ± 0.12	0.02 ± 0.00	0.07 ± 0.04	0.00 ± 0.01
liver	0.00 ± 0.00	4.40 ± 0.00	0.00 ± 0.00[c]	0.00 ± 0.40	0.10 ± 0.02	0.00 ± 0.00
muscles	0.07 ± 0.10	0.00 ± 0.00	0.10 ± 0.10	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
skin (white)	0.47 ± 0.00	0.00 ± 0.07	0.00 ± 0.17	0.04 ± 0.02	0.00 ± 0.00	0.07 ± 0.00
skin (black)	0.00 ± 0.02	0.00 ± 1.00	0.40 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.00
spleen	0.02 ± 0.00	0.40 ± 0.01	0.17 ± 0.00[c]	0.14 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
fat tissue	0.74 ± 0.70	0.00 ± 0.00	0.11 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.02
adr. glands	1 0.70 ± 0.00	0.40 ± 0.01	0.00 ± 0.00[c]	0.10 ± 0.02	0.00 ± 0.01	0.01 ± 0.00
brain	0.07 ± 0.00	0.00 ± 0.01	0.04 ± 0.00	0.00 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
testicles	0.04 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.14 ± 0.02	0.01 ± 0.00	0.00 ± 0.00

a) mean ± S.D. values of three to five determinations in 2 animals
b) mean ± S.D. values of three to five determinations in 1 animal
c) µg-equiv. / ml blood

Table 3

Tissue distribution of Ro 40-7592 (µg-equiv./g tissue) in pigmented female piebald rats following a single dose of 5 mg/kg of ^{14}C-labelled Ro 40-7592

Tissue / organ	Time (hours) 0.5[a]	2[a]	4[a]	7[a]	24[a]	48[a]
blood[b]	2.01 ± 0.47	1.00 ± 0.10	0.05 ± 0.00	0.00 ± 0.10	0.02 ± 0.00	0.00 ± 0.00
heart	1.00 ± 0.00	0.40 ± 0.02	0.00 ± 0.10	0.10 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
lungs	1.00 ± 0.10	0.00 ± 0.00	0.02 ± 0.01	0.00 ± 0.02	0.01 ± 0.00	0.04 ± 0.00
stomach	04.70 ± 17.00	7.04 ± 0.01	10.07 ± 2.02	11.10 ± 10.17	1.00 ± 1.00	0.00 ± 0.01
intestine	10.74 ± 0.44	4.07 ± 0.00	44.70 ± 04.00	00.00 ± 07.00	0.14 ± 1.00	1.02 ± 0.01
kidney	4.01 ± 0.70	0.77 ± 0.00	2.00 ± 0.01	2.02 ± 0.04	0.11 ± 0.01	0.00 ± 0.00
liver	0.77 ± 1.74	4.00 ± 0.10	4.00 ± 0.00	0.10 ± 0.00	0.10 ± 0.01	0.00 ± 0.00
muscles	0.00 ± 0.10	0.00 ± 0.01	0.10 ± 0.00	0.00 ± 0.01	0.04 ± 0.00	0.00 ± 0.00
skin (white)	1.10 ± 0.77	0.00 ± 0.00	0.00 ± 0.00	0.14 ± 0.01	0.00 ± 0.01	0.00 ± 0.00
skin (black)	1.00 ± 0.07	0.00 ± 0.07	0.00 ± 0.04	0.17 ± 0.02	0.00 ± 0.00	0.02 ± 0.00
spleen	4.40 ± 0.40	0.00 ± 0.01	0.00 ± 0.02	0.10 ± 0.00	0.01 ± 0.01	0.01 ± 0.00
fat tissue	0.01 ± 0.00	0.12 ± 0.01	0.12 ± 0.04	0.00 ± 0.02	0.01 ± 0.01	0.00 ± 0.00
adr. glands	0.02 ± 0.00	0.10 ± 0.04	0.02 ± 0.00	0.10 ± 0.04	0.00 ± 0.11	b.d.
brain	0.11 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	b.d.	b.d.
uterus	1.21 ± 0.01	0.40 ± 0.10	0.04 ± 0.01	0.20 ± 0.04	0.01 ± 0.01	b.d.

a) mean ± S.D. values of three to five determinations in 2 animals
b) mean ± S.D. values of three to five determinations in 1 animal
c) µg-equiv. / ml blood
b.d. = below detection limit

D.4. Tissue distribution following single oral administration of ^{14}C-Tolcapone (20 mg/kg) to albino rats

Research Report #: J-146,485 Volume: 80

Summary:

The tissue distribution of 20 mg/kg ^{14}C-tolcapone was determined in albino Sprague-Dawley rats (15 M, 15 F) at 0.5-24 hrs after oral administration. Concentrations of radioactivity were determined by scintillation counting. Blood, brain, liver, kidney, and small intestine were further analyzed for parent and 3-O-methyltolcapone at 0.5, 2 and 7 hrs after dosing.

Maximum levels of tissue radioactivity were attained at 0.5 hr post-dose. Highest concentrations were achieved in the organs of absorption (gut) and elimination (liver, kidney, bladder). At 0.5 and 2 hrs, intact drug accounted for 40-75% of radioactivity in blood. In other tissues, however, generally less than 10% of radioactivity was tolcapone or 3-O-methyltolcapone. Minor levels of 3-O-methyltolcapone were found in liver, kidney, and small intestine, but blood levels of this metabolite were approximately equivalent to those of parent compound at 7 hrs post-dose. As in the preceding study, only low levels of radioactivity and tolcapone were detected in brain. By 7 hrs post-treatment, neither the parent compound nor the 3-O-methyl metabolite were detected in the liver or kidney (Sponsor Tables 1-4).

An *ex vivo* plasma protein binding study confirmed *in vitro* observations that tolcapone is highly bound to plasma proteins (Tables 5 & 6).

Table 1 Tissue concentration and distribution of ^{14}C-radioactivity after a single oral administration of ^{14}C-Ro 40-7592 to male rats at a dose of 20 mg/kg

TISSUE	0.5hr µg eq/g tissue MEAN ± S.E.	0.5hr % of dose MEAN ± S.E.	2hr µg eq/g tissue MEAN ± S.E.	2hr % of dose MEAN ± S.E.	7hr µg eq/g tissue MEAN ± S.E.	7hr % of dose MEAN ± S.E.	24hr** µg eq/g tissue MEAN ± S.E.	24hr** % of dose MEAN ± S.E.
BLOOD	13.33 ± 1.22	3.76 ± 0.32	11.14 ± 1.32	2.77 ± 0.37	0.46 ± 0.06	0.16 ± 0.02	0.17 ± 0.02	0.04 ± 0.00
BRAIN	0.47 ± 0.11	0.02 ± 0.00	0.31 ± 0.02	0.01 ± 0.00	N.D.	N.D.	0.04 ± 0.02	0.00 ± 0.00
THYMUS	1.81 ± 0.30	0.03 ± 0.00	1.16 ± 0.08	0.01 ± 0.00	N.D.	N.D.	0.03 ± 0.02	0.00 ± 0.00
LIVER	23.15 ± 3.16	3.53 ± 0.44	23.36 ± 2.08	3.04 ± 0.32	3.08 ± 0.31	0.23 ± 0.03	0.32 ± 0.05	0.00 ± 0.01
KIDNEY	38.26 ± 4.34	1.65 ± 0.20	28.23 ± 1.31	1.27 ± 0.08	5.91 ± 0.40	0.27 ± 0.01	1.20 ± 0.11	0.04 ± 0.00
HEART	4.52 ± 0.50	0.09 ± 0.01	3.06 ± 0.27	0.07 ± 0.00	0.19 ± 0.02	0.00 ± 0.00	N.D.	N.D.
LUNG	6.06 ± 1.14	0.14 ± 0.03	3.09 ± 0.30	0.08 ± 0.01	0.30 ± 0.04	0.01 ± 0.00	N.D.	N.D.
STOMACH	1111.36 ± 23.89	40.80 ± 4.80	472.41 ± 164.91	19.40 ± 6.21	3.47 ± 2.00	0.58 ± 0.34	0.47 ± 0.41	0.11 ± 0.00
SMALL INTESTINE	23.04 ± 4.80	3.15 ± 0.51	90.28 ± 3.34	10.36 ± 1.01	0.72 ± 1.30	1.13 ± 0.15	1.40 ± 0.47	0.23 ± 0.00
SMALL INTESTINE CONTENTS		9.53 ± 1.50		36.94 ± 4.85		8.06 ± 0.86		1.51 ± 0.00
LARGE INTESTINE	2.58 ± 0.63	0.23 ± 0.02	4.39 ± 0.70	0.34 ± 0.04	218.43 ± 30.42	25.12 ± 2.65	17.77 ± 0.82	3.08 ± 0.00
SPLEEN	2.60 ± 0.30	0.03 ± 0.00	1.70 ± 0.12	0.02 ± 0.00	0.14 ± 0.01	0.00 ± 0.00	N.D.	N.D.
PANCREAS	3.08 ± 0.68	0.05 ± 0.01	4.30 ± 1.08	0.07 ± 0.02	0.24 ± 0.06	0.00 ± 0.00	N.D.	N.D.
PITUITARY GLAND	3.94 ± 0.57	0.00 ± 0.00	3.31 ± 0.61	0.00 ± 0.00	N.D.	N.D.	N.D.	N.D.
THYROID GLAND	3.92 ± 0.62	0.00 ± 0.00	3.43 ± 0.30	0.00 ± 0.00	0.86 ± 0.13	0.00 ± 0.00	0.90 ± 0.53	0.00 ± 0.00
ADRENAL GLAND	3.85 ± 1.04	0.01 ± 0.00	2.63 ± 0.41	0.00 ± 0.00	0.57 ± 0.05	0.00 ± 0.00	N.D.	N.D.
EYE BALL	0.84 ± 0.16	0.00 ± 0.00	0.59 ± 0.14	0.00 ± 0.00	0.11 ± 0.02	0.00 ± 0.00	N.D.	N.D.
CARCASS*	0.66 ± 0.11	9.86 ± 1.40	0.52 ± 0.05	7.61 ± 0.67	0.38 ± 0.05	1.06 ± 0.19	0.02 ± 0.00	0.27 ± 0.01
MESENTERIC LYMPH NODE	3.44 ± 0.49		3.18 ± 0.39		0.34 ± 0.05		0.06 ± 0.03	
BROWN FAT PAD	3.83 ± 0.64		3.07 ± 0.40		0.31 ± 0.06		N.D.	
WHITE FAT PAD	1.65 ± 0.31		1.54 ± 0.33		0.15 ± 0.04		N.D.	
SKIN	2.67 ± 0.44		2.61 ± 0.19		0.30 ± 0.06		0.07 ± 0.04	
MUSCLE	1.38 ± 0.25		0.94 ± 0.05		0.10 ± 0.02		N.D.	
BONE	2.59 ± 0.49		2.21 ± 0.14		0.13 ± 0.02		0.06 ± 0.02	
BLADDER	24.46 ± 10.73	0.07 ± 0.00	33.14 ± 2.08	0.05 ± 0.01	3.03 ± 1.30	0.01 ± 0.00	0.18 ± 0.02	0.00 ± 0.00
TESTIS	1.24 ± 0.16	0.04 ± 0.01	3.02 ± 0.18	0.10 ± 0.01	0.26 ± 0.07	0.00 ± 0.00	N.D.	N.D.
TOTAL		81.28 ± 1.29		83.89 ± 1.37		40.16 ± 2.61		4.37 ± 0.46

*: A whole animal after extirpation of the organs and all or part of other tissues
N.D.: Not detected
Each value represents the mean ± S.E. (n=4, **: n=3).

male rats

Table 2 Tissue concentration and distribution of ^{14}C-radioactivity after a single oral administration of ^{14}C-Ro 40-7592 to female rats at a dose of 20 mg/kg

Tissue Name	0.5hr μg eq/g tissue MEAN ± S.E.	0.5hr % of dose MEAN ± S.E.	2hr μg eq/g tissue MEAN ± S.E.	2hr % of dose MEAN ± S.E.	7hr μg eq/g tissue MEAN ± S.E.	7hr % of dose MEAN ± S.E.	24hr** μg eq/g tissue MEAN ± S.E.	24hr** % of dose MEAN ± S.E.
BLOOD	12.17 ± 4.30	3.04 ± 1.00	7.09 ± 1.32	1.73 ± 0.30	0.34 ± 0.00	0.13 ± 0.02	N.D.	N.D.
BRAIN	0.39 ± 0.07	0.00 ± 0.00	0.33 ± [illegible]	0.00 ± 0.00	N.D.	N.D.	N.D.	N.D.
THYMUS	1.65 ± 0.41	0.03 ± 0.01	1.00 ± 0.19	0.02 ± 0.00	0.27 ± 0.05	0.00 ± 0.00	N.D.	N.D.
LIVER	20.63 ± 4.63	3.17 ± 0.71	15.03 ± 2.33	2.37 ± 0.32	4.41 ± 0.25	0.83 ± 0.05	0.21 ± 0.03	0.05 ± 0.01
KIDNEY	16.36 ± 3.32	0.73 ± 0.14	11.80 ± 2.43	0.51 ± 0.10	3.02 ± 0.23	0.15 ± 0.01	0.36 ± 0.04	0.02 ± 0.00
HEART	3.07 ± 1.01	0.07 ± 0.02	1.33 ± 0.36	0.03 ± 0.01	0.19 ± 0.03	0.00 ± 0.00	N.D.	N.D.
LUNG	4.11 ± 1.01	0.10 ± 0.03	2.93 ± 0.29	0.07 ± 0.01	0.28 ± 0.03	0.01 ± 0.00	0.05 ± 0.05	0.00 ± 0.00
STOMACH	997.00 ± 60.83	32.93 ± 3.47	198.43 ± 85.31	7.37 ± 2.98	3.14 ± 2.51	0.73 ± 0.30	0.27 ± 0.13	0.03 ± 0.01
SMALL INTESTINE	33.94 ± 7.09	4.33 ± 1.08	60.15 ± 3.79	8.81 ± 0.76	7.40 ± 1.08	1.07 ± 0.20	1.43 ± 0.34	0.23 ± 0.05
SMALL INTESTINE CONTENTS		14.40 ± 2.34		42.36 ± 4.74		9.90 ± 1.52		2.21 ± 0.58
LARGE INTESTINE	1.64 ± 0.30	0.22 ± 0.08	25.77 ± 12.73	2.80 ± 1.33	390.35 ± 33.99	60.51 ± 3.42	36.66 ± 15.23	8.79 ± 3.68
SPLEEN	2.03 ± 0.60	0.03 ± 0.00	1.30 ± 0.34	0.00 ± 0.00	N.D.	N.D.	N.D.	N.D.
PANCREAS	3.63 ± 1.09	0.06 ± 0.02	1.67 ± 0.34	0.03 ± 0.01	0.43 ± 0.12	0.01 ± 0.00	0.12 ± 0.00	0.00 ± 0.00
PITUITARY GLAND	3.01 ± 1.00	0.00 ± 0.00	1.05 ± 0.30	0.00 ± 0.00	N.D.	N.D.	N.D.	N.D.
THYROID GLAND	2.38 ± 0.57	0.00 ± 0.00	1.59 ± 0.40	0.00 ± 0.00	0.40 ± 0.10	0.00 ± 0.00	N.D.	N.D.
ADRENAL GLAND	2.43 ± 1.50	0.01 ± 0.00	2.04 ± 0.37	0.00 ± 0.00	0.30 ± 0.03	0.00 ± 0.00	N.D.	N.D.
EYEBALL	0.34 ± 0.15	0.00 ± 0.00	0.73 ± 0.09	0.00 ± 0.00	0.06 ± 0.00	0.00 ± 0.00	N.D.	N.D.
CARCASS*	1.98 ± 0.61	7.03 ± 2.40	0.53 ± 0.04	4.34 ± 0.50	0.11 ± 0.02	1.53 ± 0.27	0.04 ± 0.01	0.50 ± 0.08
MESENTERIC LYMPH NODE	2.59 ± 0.73		2.73 ± 0.35		0.30 ± 0.10		0.13 ± 0.06	
BROWN FAT PAD	2.96 ± 0.91		2.30 ± 0.40		0.17 ± 0.02		N.D.	
WHITE FAT PAD	1.94 ± 0.41		0.84 ± 0.12		0.10 ± 0.01		N.D.	
SKIN	2.33 ± 0.47		1.03 ± 0.08		0.37 ± 0.08		N.D.	
MUSCLE	1.08 ± 0.44		0.74 ± 0.11		0.08 ± 0.01		N.D.	
BONE	1.75 ± 0.60		1.07 ± 0.08		N.D.		N.D.	
BLADDER	14.53 ± 6.09	0.00 ± 0.01	3.23 ± 1.04	0.01 ± 0.00	2.23 ± 1.09	0.00 ± 0.00	0.22 ± 0.07	0.00 ± 0.00
UTERUS	3.05 ± 1.30	0.04 ± 0.02	3.05 ± 0.38	0.04 ± 0.01	0.27 ± 0.01	0.00 ± 0.00	N.D.	N.D.
OVARY	4.09 ± 1.30	0.01 ± 0.00	2.93 ± 0.57	0.01 ± 0.00	0.35 ± 0.00	0.00 ± 0.00	N.D.	N.D.
TOTAL		85.07 ± 3.80		70.73 ± 1.41		74.86 ± 3.58		11.90 ± 3.34

* : A whole animal after extirpation of the organs and all or part of other tissues
N.D.: Not detected
Each value represents the mean ± S.E. (n=4, ** : n=3).

female rats

Table 3 · Tissue concentration of intact drug and its metabolite (Ro 40-7591) after a single oral administration of ^{14}C-Ro 40-7592 to male rats at a dose of 20 mg/kg

		LIVER 0.5hr	LIVER 2hr	LIVER 7hr	KIDNEY 0.5hr	KIDNEY 2hr	KIDNEY 7hr
Ro 40-7592	μ g eq/g tissue	1.14 ± 0.23	1.04 ± 0.23	N.D.	0.44 ± 0.07	0.60 ± 0.13	N.D.
	% of ^{14}C-radioactivity	4.85 ± 0.39	4.34 ± 0.63	N.D.	1.16 ± 0.06	2.31 ± 0.32	N.D.
Ro 40-7591	μ g eq/g tissue	0.06 ± 0.01	0.09 ± 0.01	N.D.	N.D.	N.D.	N.D.
	% of ^{14}C-radioactivity	0.24 ± 0.05	0.38 ± 0.08	N.D.	N.D.	N.D.	N.D.
Total	% of ^{14}C-radioactivity	5.08 ± 0.45	4.72 ± 0.61	N.D.	1.16 ± 0.06	2.31 ± 0.32	N.D.

		SMALL INTESTINE 0.5hr	SMALL INTESTINE 2hr	SMALL INTESTINE 7hr	BRAIN 0.5hr	BRAIN 2hr	BRAIN 7hr
Ro 40-7592	μ g eq/g tissue	0.71 ± 0.21	4.74 ± 0.44	N.D.	N.D.	N.D.	N.D.
	% of ^{14}C-radioactivity	2.98 ± 0.44	5.29 ± 0.54	N.D.	N.D.	N.D.	N.D.
Ro 40-7591	μ g eq/g tissue	N.D.	0.13 ± 0.02	N.D.	N.D.	N.D.	N.D.
	% of ^{14}C-radioactivity	N.D.	0.14 ± 0.02	N.D.	N.D.	N.D.	N.D.
Total	% of ^{14}C-radioactivity	2.98 ± 0.44	5.43 ± 0.54	N.D.	N.D.	N.D.	N.D.

		BLOOD 0.5hr	BLOOD 2hr	BLOOD 7hr
Ro 40-7592	μ g eq/g tissue	6.42 ± 1.23	5.66 ± 1.94	0.16 ± 0.04
	% of ^{14}C-radioactivity	41.14 ± 1.82	55.97 ± 17.19	22.93 ± 3.79
Ro 40-7591	μ g eq/g tissue	0.30 ± 0.10	0.27 ± 0.10	0.17 ± 0.04
	% of ^{14}C-radioactivity	1.85 ± 0.39	2.93 ± 1.27	25.36 ± 4.22
Total	% of ^{14}C-radioactivity	43.16 ± 2.11	58.90 ± 18.02	48.29 ± 5.83

N.D.: Not detected
Each value represents the mean ± S.E. (n=4).